

nature

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Scientists think, too

IMAGES of science and the scientist and of engineering and the engineer are much discussed at present. The most recent issue of *Physics Today* contains W. A. Fowler's Presidential Address to the American Physical Society in which some ideas on brushing up the physicist's image are put forward. British engineers, deeply concerned at what they believe is society's lowly rating of them, have been puzzling for some time over how they can better present their profession. And the Spring issue of *Science for People*, the magazine of the British Society for Social Responsibility in Science, is devoted to images of science; we are told that scientific work, being subject to an extreme division of labour, 'tends to be boring, unfulfilling, alienating and unhealthy like most jobs in a capitalist society . . . Being a scientific worker means working in a patriarchal, repressive hierarchy, often in bad working conditions, without control over the fruits of one's labour'.

Images are, of course, needed for a variety of purposes. The student will see the scientific profession in a certain light and will accordingly decide whether or not to contemplate a scientific career. The industrial employer will have a different sort of perception of science related to value to the company. The politician will want to know whether scientists and technologists are basically trustworthy in their advice or whether they are prone to empire building and self-serving pronouncements. The layman, relying more and more on science and technology to deliver the goods, will have his or her own view of whether the scientist and technologist substantiate their claims. Occasionally in all of these directions we can be pleasantly surprised at the reservoir of goodwill, trust and interest which remains.

But there is one image which is in danger of being submerged amidst the recent frenzy to project science as still valuable and scientists as (in Fowler's words) capable, practical, ingenious, innovative and at the same time human beings. That is the image of scientists as thinkers. The public still remembers Einstein and recognises him as a great thinker, but is science in general considered to be a thinking profession? Of

course this image is not necessarily one which will be for mass-consumption, but it is an important one nevertheless, because other people in our society most certainly regard themselves as thinkers and it is important that they do not dismiss scientists as doers only, and therefore of not being capable of participating in sensible general discussions on broad matters of policy. This has happened to a large extent in Britain already, with precious little action from bodies which are supposed to have the scientist's interest at heart. There is deplorably little involvement of the scientist in what might be called 'the life of the nation' and the process is, of course, self-reinforcing: the more the exclusion, the smaller the number of those who can talk sense.

What to do to establish footholds? Clearly anything involving the expenditure of large sums is not going to be feasible in the near future, so we may have to rule out such desirable ideas as seconding scientists to work with politicians, or the establishment of new interdisciplinary institutions. There still remain, however, opportunities within the media—the arch-purveyors of image. Last year there was great satisfaction that a young biologist should be asked to deliver the BBC's Reith Lectures. But very few scientists are allowed to express their own views in any extended way either on work they are involved in themselves or on more general matters. The formula is generally that scientists, if allowed to speak or write at all for the general public, do so almost invariably through short extracts only, with a journalist or reporter on hand to explain what the scientist means. There is nothing fundamentally wrong with this format (not that many professors in the humanities would stand for it), but in the absence of more extended contributions in the columns of serious newspapers, in magazines and in radio talks, the impression is undoubtedly left that scientists aren't really capable of stringing together more than a few dozen words, are only worth hearing on their very narrow speciality and are best not disturbed in their busy life of doing things. It is time the thinking side of science was given a more public airing. □



Chemical disarmament still elusive

THE Spring session of the United Nations Conference of the Committee on Disarmament (CCD) droned to a close in Geneva this month with little concrete progress made on the thorny issues involved in negotiating a chemical disarmament treaty. Though a proposal put forward last year by the British delegation received many general endorsements—including one from the United States—delegates have been marking time for the past three years awaiting a long-promised but elusive joint proposal from the United States and the Soviet Union.

Part of the problem stems from the fact that there is considerable confusion about the United States position, particularly in regard to the US Army's plans to launch a massive new programme to replace its existing chemical weapons stockpiles with so-called binary chemical weapons. The time has come for President Carter to clarify the matter.

The background is this. In the early 1970s, while US delegates were telling the CCD that the United States supports chemical disarmament, the US Army was requesting funds from Congress to begin building binary weapons—nerve agents made from two non-lethal components which form a deadly compound when mixed together. Not surprisingly, the negotiators were not taken very seriously. In 1974, however, Congress refused to appropriate the funds. Undeterred, the Army came back with another request

the following year. This time, the House Armed Services Committee deleted the funds, but it stated that unless progress is made at the CCD talks, future requests for binary weapons would be viewed more favourably.

Consequently, no funds were requested for binaries in the budget proposed by President Ford early last year, but the Army nevertheless argued within the Administration for the programme to be reinstated in President Ford's final budget, presented to Congress last January. Ford decided not to include the funds, however, and passed the matter on to his successor. The binary programme is therefore far from being killed off, and it still looms large over the CCD talks.

While this was going on, President Nixon and Secretary Brezhnev announced at their summit meeting in July 1974 that the United States and the Soviet Union would offer a joint initiative to break the deadlock at the CCD talks. So far, however, only two rounds of bilateral discussions have been held, the last of which took place early in April, and little progress was made.

Very tricky issues concerned with measures to police a chemical disarmament treaty are involved in the negotiations. But the time has come at least for President Carter to clear the air by announcing that the United States will scrap its plans to produce binary weapons and that the new Administration is serious about chemical disarmament.

The microprocessor phenomenon

Basil Zacharov separates fact and fiction to comment on the microprocessor 'revolution'

SINCE the introduction of the first commercial microprocessor in 1971 by INTEL the growth in number of microprocessors has been little short of phenomenal. It is estimated that the present sales, already £10 million for the European market, will grow at least tenfold by 1980. Microprocessors are used in scientific, domestic, military and commercial applications. Conferences entirely devoted to microprocessors are now commonplace. There are professional journals which are concerned only with microprocessors. And there is hardly any research grant application with a computing or electronic content where the word 'microprocessor' does not appear somewhere or other.

The microprocessor is an electronic device, fabricated on a single small semiconductor chip, enabling certain operations to be performed on input digital data, the transformed data then becoming available either on an output data highway, or in appropriate internal registers of the microprocessor. Depending upon the choice of microprocessor, the width of the data path may be anything from 1 bit upwards, and the operations may include arithmetic instructions, logical instructions and certain others, familiar to every professional computer programmer, such as conditional and interrupt-handling operations. Thus the microprocessor is just one example of a very broad class of monolithic digital elements, using one of several large-scale integration (LSI) technologies to implement as many as 10^5 components on a single semiconductor chip perhaps only 25 mm² in area.

Not surprisingly perhaps, the very rapid evolution and exploitation of microprocessors has been accompanied by a corresponding growth in publicity, some of which has been not altogether accurate. There has emerged a mythology about microprocessors or, at the least, a certain amount of confusion. Probably the most widespread myth is that a

microprocessor is a microcomputer which, still, it patently is not. Probably one should not be too pedantic about this, and anyway there are many different kinds of stored-programme digital computer, but all of them have to have a memory, a control unit for instruction sequencing and some means of input and output as well as the processing section. So the microprocessor is nothing more than one component of a computer although, admittedly, a very powerful component indeed.

A much more dangerous myth is that it is easy to replace minicomputers by microprocessor systems and presumably, because the cost of microprocessors is so low, that it is possible to build a microprocessor-based minicomputer at a cost much lower than that of commercially-available minicomputers. And so we have seen numerous examples of minicomputers fabricated by the do-it-yourself process. The truth, unfortunately, is that, if all the component costs are really taken into account (including power supplies, circuit cards, casing and so on) and fabrication costs properly included, then it is almost impossible to compete even with the hardware costs of small minicomputers supplied to equipment manufacturers (the so-called 'naked' minicomputers, with no peripherals or other trappings). And this is not the least surprising, for minicomputer manufacturers have been making use of modern LSI components just as much as anyone else. However, if the fact is included that even the most modest minicomputer arrives with some software, then any thought that an individual can compete economically in synthesising a minicomputer just cannot be supported.

A more subtle myth is the claim that, because microprocessors are inexpensive, it is possible to produce at much lower cost a computing system that is comparable in performance to that of large general-purpose computers. Such claims are generally supported by demonstrating that microprocessor-based computing systems can be built with certain instruction execution times comparable to those of some chosen large computer. The more outspoken of those making these claims then go on to conclude that anyone

using the large computer on a shared basis would do well to abandon this enslavement and turn instead to a 'personal' computer based on microprocessors. The millennium has, unfortunately, not yet arrived, and the reason is of course clear. It is simply that, although it is indeed possible to achieve comparable execution times for certain operations, as for example adding two integers, this is just hopelessly inadequate to achieve the performance of a large computer for calculations of a general nature.

Large computers achieve their performance precisely because of their very extensive repertoire of operations, and also because of their complex internal organisation which allows many different concurrent activities. If this performance is required, then the complexity, and hence cost, of the large system is inescapable. That is not to say that the architecture of modern large computers is the best that can be imagined, nor that a large computer is best for all purposes; just that modern large computers are fairly well matched to the requirements of most large data-processing and scientific calculations, for which a microprocessor-based alternative would be inadequate.

Of course there may be certain special computational tasks that only involve just those operations that can be implemented well with microprocessors; in this case it may well be the best choice to use the microprocessor system. But, even here, the problem has to be one which occurs frequently—otherwise it may still be much more economical to use a conventional computer inefficiently. It must be emphasised here that, just as in the case of minicomputers, large systems use LSI techniques where they can and if they are costly, it is because of all the general facilities that they possess. Microprocessors have no monopoly of LSI technology.

So what is it that microprocessors offer that has led to such an upsurge in their numbers? The most important attributes appear to be small size and low cost, but these relate to the microprocessor as a digital systems component, and not to such things as complete computers. And the advantages in using microprocessors stem from the fact that these attributes enable them to be used either where an application requires large numbers of processors or where processors need to be built into some device or other. Moreover, in applications where the processing task is completely defined and does not need to be changed, there can be a very significant economic advantage in using a microprocessor system tailored to the task rather than use a more versatile minicomputer. The advantages of microprocessors when used in these 'turnkey' systems increase tremendously when the number of similar systems is great. Such problems had been tackled in the past by building special-purpose electronic systems, but these were generally expensive and very difficult to modify. Microprocessors are not only much less costly for these purposes, but also more reliable and, more important, they allow easy evolution from one system to another by changing software.

The real benefit of microprocessors has appeared in computer peripheral devices, especially terminals, and in instrumentation of all kinds. So we have seen a dramatic change already in data presentation from instruments to human beings, and in every application where electronic systems have to respond in an adaptive way to external conditions. This introduction of so-called 'intelligence' into electronic systems has transformed the operation of a wide range of systems, from oscilloscopes to washing machines, from missiles to traffic lights, from computer terminals to automobile electronics. What was costly and complicated to do before using conventional hard-wired electronic components has now become inexpensive and relatively simple to implement.

This trend towards replacement of hard-wired electronic logic by software-programmed microprocessor-based

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systems is nowhere more apparent than in digital communications, itself one of the most rapidly growing technological fields. Already the use of microprocessors in coupling remote devices to computer networks is commonplace, but we are witnessing only the beginning of this trend. However, all these applications generally involve the use of only a few LSI devices for each unit of equipment, and we need also to remember the other area mentioned, where many microprocessors are used together.

For many years now there has been the idea that what is difficult to do on a conventional sequential-processing computer might be much easier to do on an array of many coupled processors all operating in parallel. Computational problems which either have intrinsic parallelism or can be suitably formulated in terms of some parallel algorithms exist in many different fields, but their solutions have been impeded by the lack of appropriate hardware. Although attempts have been made to implement parallel computer systems using conventional hardware, these attempts have been immensely costly. Now, with the advent of microprocessors, the construction of parallel processing systems has become a real economic prospect and, together with it, the promise of effective solutions to a whole new class of problems.

So, although there is a mythological element in what is being claimed for microprocessors which sometimes distracts certain sectors from the realities of the situation, it is no myth that microprocessors have made a most fundamental impact on many different fields. Microprocessors are already transforming both the construction and the exploitation of an immense range of instruments, appliances and systems, and the signs are that this trend is only a beginning. But perhaps it is as well to end on a note of caution and to mention a final myth—namely that microprocessors are easy to use, and that it is just as easy for hardware designers to employ them as it was for them to design circuits using conventional integrated circuits. What is overlooked here is of course software, which many circuit designers are seeing for the first time.

It is probably the case that, except for a few talented individuals or for very trivial tasks, it was never particularly easy to program a computer effectively in machine code, and not only are microprocessors no exception here but they are also rather primitive. The solution to this problem is well-enough understood, for example by using what are termed high-level techniques to insulate the microprocessor system user or designer from all the details of particular system components. This solution will take a long time to establish since it depends upon agreement about standards and conventions, among other factors, and these do not yet exist. However, even with high-level techniques, it will still be necessary for hardware designers to be familiar with software. Indeed, they will no longer be circuit designers but rather systems engineers. For this to come about, and for the full potential of microprocessors to be realised, we will have to adapt our educational system to meet the new needs of such systems engineers. □

Freedom for whales

The great whales need protection from man.
Ray Gambell describes the efforts to provide it

THE control of whaling by the International Whaling Commission (IWC), which regulates about 90% of whaling activity throughout the world, has changed very considerably in recent years. There is now a long term management policy aimed at bringing all whale stocks to their most productive levels. Stocks which have been reduced below these levels in the past are given total protection from hunting so that they can rebuild at the fastest possible rate. All management decisions are now based only on the best scientific advice available on the condition and sustainable harvesting capacities of the stocks. Previously the economic arguments of the whaling industry weighed very heavily in decisions taken on the number of whales which were allowed to be caught and the stocks were successively overtaxed to varying degrees.

This major change in management policy started in 1965 but developed more rapidly after the 1972 UN Conference on the Human Environment in Stockholm at which a three-part resolution on whaling was adopted. It called for the strengthening of the IWC, an increase in international research on whales, and a ten-year moratorium on commercial whaling.

Ever since the IWC was set up by the International Convention for the Regulation of Whaling, signed in 1946, the secretariat had been provided on a part time basis by the UK Ministry of Agriculture, Fisheries and Food. In recent years it became clear that the part-time secretariat had difficulty in meeting the increasing demands placed upon it and so in 1976 the Commission established a full-time secretariat headed by an experienced whale biologist. The offices of the Commission have now moved from London to Cambridge, where there are a number of research institutions concerned with whales and other marine mammals. The secretariat is now able to devote itself completely to the development of the Commission's activities, including publicity, publications and the general servicing of the Commission's day-to-day requirements.

International research

A great deal of current knowledge of the biology, life history and status of the stocks of the great whales has been gained with the generous cooperation

of the whaling industry itself. Biologists have been able to collect and analyse materials from the whale carcasses during normal commercial operations and facilities for sighting and marking programmes, which would not otherwise have been undertaken have been provided. Inevitably, with the reduction of the whaling industry in recent years, the scope for research based on commercial operations has declined. This is particularly noticeable in terms of the sighting areas covered by the fleets, which now provide the only records of the protected species.

Whale research on an adequate scale for proper global management is very expensive. In 1975 the Scientific Committee of the IWC put forward detailed proposals known as the International Decade of Cetacean Research (IDCR), for research on the distribution and status of the stocks of whales in the southern hemisphere, North Pacific, and North Atlantic Oceans. They have been submitted to the United Nations Environment Programme as one possible funding agency. It is estimated that the first year of this ten-year programme will cost \$2.4 million at 1974 prices and, so far there has been no progress in obtaining funding of this magnitude. The delay has been partly brought about by a number of bodies awaiting the research proposals from the Food and Agriculture Organisation (FAO) Mammals in the Sea Scientific Consultation held in Bergen in September 1976.

In the meantime the IWC has set up its own voluntary research fund. Only a few contributions have been received so far, but it is hoped that a number of the IDCR proposals can be funded and implemented through this research fund. In addition, of course, the various national research groups continue their own programmes.

When the IWC considered the call for a total moratorium on commercial whaling it accepted the advice of its Scientific Committee that such action could not be justified scientifically since prudent management required regulation of each stock individually. Nevertheless, the Commission did formulate and adopt precise guidelines for the control of whaling based on the concept of Maximum Sustainable Yield (MSY). All stocks of whales are now classified into one of three categories according to the advice of the Scientific Committee:

- Initial Management Stocks, which may be reduced in a controlled manner

to achieve MSY levels or optimum levels as these are determined.

- Sustained Management Stocks which will be maintained at or near MSY levels and then at optimum levels as these are determined.

- Protection Stocks, which are below the level of sustained management stocks and will be fully protected.

All whale stocks have a natural capacity for increase and a natural rate of mortality. At their initial level these two factors balance one another so that the stock remains more or less in equilibrium. As the stock is reduced the pregnancy rate rises, the age at which the whales start to reproduce falls and recruitment increases. At some particular stock level the surplus of recruits over natural deaths reaches a maximum—the MSY—which can be safely harvested without reducing the stock size. At stock sizes below this level the surplus of recruits over natural mortalities declines until another equilibrium position is reached at very small stock levels.

The Scientific Committee has responsibility for identifying stocks of all species of large whales and classifying them according to these categories. The classification is revised annually. The size of catch which may be taken from the stocks is then determined more or less from automatic rules. If a stock is to be judged to be substantially smaller than the MSY level total protection is declared. If it is larger, catching is permitted under quotas which should allow a controlled reduction of the stock towards the MSY level. If the stock is already near the MSY level annual quotas are set with the intention of maintaining the stock at about that size. The rules for determining catch limits allow for possible errors in MSY estimates. For example, no more than 90% of the estimated MSY can be taken whatever the classification of the stocks. This gives a margin of 30% either way in the estimation of the MSY stock level with a symmetrical yield curve. There are also supplementary rules for determining the rates of catch when there is insufficient knowledge to assess the status of the stock, especially when exploitation has only just begun, and also when a stock has apparently been exploited at a sustainable level for many years.

Scientific advice

Despite the advances made by the IWC in bringing its house into order in recent years, there is still considerable public interest and debate over the way in which the whale stocks are harvested. One area of criticism concerns the activities of the Scientific Committee itself. There is much discussion

over the concept of MSY as a basis for management. The Scientific Committee is very much aware of this problem and is open to any proposals which would help it in its task of assessing, classifying and offering advice on the regulation of the whale stocks. For the moment, however, the MSY concept is one which does give practical guidelines for the management of the whaling industry which can be kept under constant review. It also allows for new factors to be introduced to develop the idea of optimum stock levels.

The International Union for the Conservation of Nature and Natural Resources has called for the replacement of the MSY concept by more ecologically based criteria but unfortunately, it is not yet possible to quantify such a basis for management. Discussions are going on between the IWC Scientific Committee and the IUCN with a view to developing these ideas.

A further source of difficulty is the extent to which the Scientific Committee's activities and discussions are available for public scrutiny. Because it meets in the two weeks immediately preceding the Commission's Annual Meeting held in June every year, there is no possibility of publishing the reports of its work before the meeting. This has happened because the Committee must have the latest catch statistics from the Antarctic whaling season which finishes only a few weeks earlier. However, all the scientific papers contributing to management decisions are available immediately after the meeting, and most of them are published subsequently in the Commission's Annual Report.

The Commission has been criticised

as a club for exploiters but the majority of the sixteen member nations do not catch whales commercially. Also, any government may adhere to the 1946 Convention and so play a full role in the conservation and management of the whale stocks. When the Commission was first set up it recognised that specialised agencies related to the United Nations would also be concerned with the conservation and development of whale fisheries. It therefore agreed to decide within two years of the Convention's enforcement whether it too should become a UN agency. In 1950 it resolved that it should remain an independent body, but at the same time decided to maintain its close association with the FAO. The question of IWC affiliation to the UN has not since been raised within the Commission.

The 1946 Convention is currently being reviewed and a meeting is being planned of plenipotentiaries from all significant whaling countries or countries with stocks of cetaceans off their coasts to elaborate a new cetacean convention. It is very important that all whaling should be under unified international control, for if unregulated catching goes on there is little prospect for the survival of whales as a valuable resource and component of the marine ecosystem.

Although only some 10% of the world's whaling is carried out by nations which are not members of the IWC, their catch represents all or a major part of the whaling activity in certain parts of the world. For example, off the coasts of Chile and Peru these two nations are the only countries actively whaling, while the Spanish and

Portuguese catches in the temperate Atlantic are the only ones in that area. In addition, catches by Korea and a Somali registered vessel overlap with IWC members' operations. The IWC has noted that these activities are seriously weakening its conservation measures and it has repeatedly called on these countries to adhere to the 1946 Convention. So far, diplomatic approaches by individual governments and by the United Nations and various of its specialised agencies have met with a negative response.

International inspection

The Commission's regulations are enforced by whaling inspectors appointed by the flag nation of each catching operation. They report on any infractions and carry out prosecutions through their national jurisdiction. The Commission has also established an International Observer Scheme, whereby observers are exchanged between nations which are in or operate within the same geographical areas. Thus, Japan and the USSR exchange observers on their factory ships in the Antarctic and in the North Pacific. Land station operations in Japan receive observers from the United States, and the Icelandic whaling station has a Canadian or Norwegian observer. A similar exchange is planned between Australia and Brazil, the two Southern Hemisphere land station operations with the IWC.

The observers report directly to the Commission on the manner in which the whaling operations and the inspection schemes are carried out and check that the various regulations governing whaling activities are properly implemented. It has been argued that such an interchange between nations with common interests is not sufficiently independent and that observers from non-whaling nations should be placed at the various whaling factories. This is a matter for international agreement but it must be recognised that the present observer schemes are a significant step forward in the control of the whaling industry on a global scale.

The history of the regulation of whaling this century is generally one of failure. A number of stocks have been seriously depleted in every phase of whaling activity, for whales can be caught more quickly than they are able to reproduce. Nevertheless, the recent advances in the organisation and actions of the IWC make the future look more hopeful. Although there are still many difficulties and many problems of a scientific and administrative nature the present direction of the IWC and its stated intention to manage the stocks in a responsible fashion give cause for future optimism. □

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Now, an implementation problem

Robert Stein writes on the United Nations Water Conference which took place last month

THE UN Water Conference finished its work on 25 March in Mar del Plata, Argentina, and the ripple was slight. Most of the reports coming out of the conference said nothing much happened, which was taken by reporters on the scene to be either good or bad. That no serious political controversy disrupted this conference in the way that political issues tore apart the end of the UN Human Settlements Conference in Vancouver last June was certainly positive. There was a lot of serious discussion both by those with technical expertise and those concerned with the political implications of the various uses of water. On the negative side, the lack of exciting controversy did not result in very many concrete advances, and this is seen as a failure of the conference since the problems are still waiting to be addressed.

But what was the conference trying to do—and did it come at all close to accomplishing its purposes? The representatives who journeyed many thousands of miles to participate in the conference—over one thousand of them from 116 states—had been given documentation which contained both good and bad news. There was enough water globally but, as Gilbert F. White put it in the basic UN background paper, "Frustratingly, it tends to be available in the wrong place, at the wrong time, or with the wrong quality." Yet for the developed world, the bulk of the problems had until the past few years not been theirs. Pollution, yes, but there was little expectation that a UN conference could help advance their perception of the problems. Then in the summer of 1976, water shortages in northern Europe, and the lack of snow in North America the following winter woke others up to the fact that drought was not merely a problem for the Sahel. Sound water management begins at home.

At the same time this conference, ill prepared as it was (the preparatory committee meeting in January had only one day to review the draft of the plan of action), had a specific charge, provided by the UN Human Settlements: to consider targets for supply of potable water and waste disposal.

Many of the countries of the under-

developed world addressed water supply as their most pressing problem. Kenya, Tanzania, Nepal, and Pakistan all issued pleas to the conference for help. A number of African countries called for a new special fund to assist countries in water supply and waste disposal—a cry which was not answered. Instead, the conference adopted a resolution calling for a study to figure out how to finance a variety of water development projects, a study which would not exclude the possibility of a fund.

Additionally, and more importantly, the conference approved a resolution put forward by Argentina, the host government, which called upon governments needing improved water supply and waste disposal systems to prepare programmes and plans by 1980 to reach the Habitat goals of water for all rural and urban areas by 1990. These plans would then be reviewed to determine how they could best be funded. Interestingly, the problems of maintaining broken-down systems, which are usually ignored, were made an integral part of the recommendations. The resolution was much less specific on which body or organisation would help with the implementation, but that was left for the UN's Economic and Social Council to work out. The conference dealt only generally with problems of water in arid lands and drought, preferring to let the Deserts Conference, this August, tackle that issue.

Management of shared river basins contributed greatly to whatever substantive disagreements took place at the conference. The division was usually according to a country's place in the basin: 'upstream' countries which could hold back water or pollute it to the detriment of those who were 'downstream' banded together, providing such groupings as India against Bangladesh on the Ganges; Turkey against Syria and Iraq on the Euphrates; Brazil against Argentina on the Parana; and Germany, Switzerland and France against the Netherlands on the Rhine. The resulting recommendation was clearly a political compromise urging cooperation and using the waters of the basin equitably in accordance with principles of international law. Advance consultation was also watered down, with no mandatory requirements. The work of international legal bodies was also encouraged, though given past per-

formances, many disputes—acrimonious as well as peaceable—will arise before these groups conclude their work.

The conference ended positively. An attempt to introduce a statement, which most aptly would have been called the "Declaration of Mar del Platitude", was defeated; instead the recommendations themselves were enshrined. But after the delegates packed their bathing suits and stacks of documents, and left the shining sea and all the plain-clothes police behind, what is next?

UN Conferences can only be a reflection of the willingness of member states to do something. Agreeing to a fund does not necessarily provide any real money to build water supply systems or irrigation works. A conference must—and the UN Water Conference just barely did—supply tools enabling countries and international aid-giving agencies to act in specific directions if they so choose. They are not law making conferences, like the UN Conference on the Law of the Sea is meant to be, nor can they be in two weeks. The proof is in the implementation, a part of the conference process too often ignored both by governments and the UN system.

So the questions remain. How will governments react to the proposals when they are presented to the UN Economic and Social Council in July? How many governments will make efforts—which they otherwise would not have done—over the absence of the water supply programme or the provision of aid and technical assistance to those that need it? How will the conference affect programmes of international agencies such as the World Bank, WHO, FAO, WMO, UNDP, UNICEF and all the others who came to the conference to make sure they weren't cut out of the action? These will be the tests of success or failure, and though it is too early really to gauge success, the record of the international community is not a very hopeful one. □



PROFILE

Kenneth Owen on Sir Ieuan Maddock

Bread-and-butter or exotica?

A CHOICE trio gave details of the Labour Government's new Ministry of Technology at a Millbank Tower press conference in November 1964—Frank Cousins, trade union leader turned minister; C. P. Snow the author, alias Lord Snow, a junior minister; and Professor P. M. S. Blackett, deputy chairman of the minister's Technology Advisory Council. Dr Ieuan Maddock at that time was with the Atomic Energy Authority at Aldermaston. The following September he moved over to Mintech at the invitation of Dr John Adams to help get the fledgling ministry moving.

At the end of last month, six ministers and two ministries later, Sir Ieuan Maddock, FRS retired as Chief Scientist at the Department of Industry. He remains acting director of the National Physical Laboratory.

A tough, ebullient Welshman, he had needed both toughness and ebullience to steer industry and government research establishments towards what he saw as the right kinds of technology. In the process he offended both high-technology industry and the establishments: he was bruised by the expediency of politicians.

The concept of Mintech had been generated primarily by Blackett, who knew at first hand how science had been harnessed to immediate national objectives during the 1939–45 war and who believed that industry could now be regenerated by a similar approach. Maddock joined Mintech as a deputy controller. It was then a very small ministry, responsible for only four industries—electronics, computers, telecommunications and machine tools. Mintech expanded with the transfer of the engineering industry divisions of the Board of Trade in February 1966 and Maddock went on to head five industrial divisions.

Mintech also broadened in scope to deal with basic industrial structures, not just technology. One facet of this approach led to the setting-up of ICL as Britain's national computer company. Various other programmes were mounted to stimulate the application of advanced machines and techniques in industry. "By the summer of 1969", Sir Ieuan recalls, "we were really beginning to get results".

Then came a body-blow—the change of government in 1970 which

abolished Mintech and put its initiatives into reverse. "The new government classified everything we were trying to do at Mintech as 'interventionist'. The shutters came down absolutely and completely. You were guilty until you could prove yourself innocent. A lot of schemes were scrapped long before we had a chance of finding out whether they were good or bad".

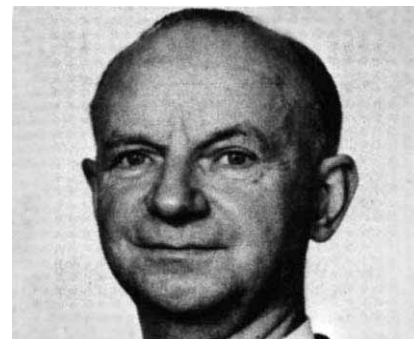
In his work as Controller of Industrial Technology at the new Department of Trade and Industry (DTI), Maddock was becoming more of an administrator, working on, among other things, the future of the steel industry. "By that time I had moved a fairly long way away from science and technology". This was rectified in February 1971, when he was appointed to the new post of Chief Scientist at the Department. He was to be responsible for the general strategy and coordination of the Department's research activities, while retaining his responsibilities for the DTI sponsorship of the steel, electronics and computer industries.

"At that time", Sir Ieuan says, "I found it personally very difficult. I was dedicated to what we had been doing at Mintech and I was seeing it dismantled. I was having to fight rearguard actions all the way, saying 'Look, you're throwing all kinds of babies away with the bathwater'—and subsequent events proved that to be right.

"I have to confess I didn't enjoy that part of my life at all. In nearly 40 years of government service it is the only period I can recall when I was really pretty unhappy with my environment".

Fresh air blew in with the Rothschild proposals, fully supported by Maddock, which introduced the customer-contractor principle into government R&D spending. "With the dip in my moral factor at that time", Sir Ieuan says, "this gave me something to get my teeth into".

The DTI itself was not a customer department, in the way that Defence was; how should the Rothschild principles be applied? Maddock's solution was to set up a group of industry-government research requirements boards to act as proxy customers, decide priorities and allocate funds. As their programmes came under scrutiny from the boards, the government laboratories hated



Sir Ieuan Maddock

the whole exercise, Sir Ieuan admits cheerfully. The balance of R&D spending changed, with less being channelled through the establishments and more through the research associations (and through industry).

On public occasions throughout his time at Mintech, DTI and DoI, Sir Ieuan has repeatedly stressed the importance of the bread-and-butter industries and the dangers of pursuing high-technology exotica. The transfer of existing technologies is more important than the creation of new ones; this has become his personal crusade.

"It's not so much the money, it's where you address the best intellects", he says. "The staff list at Harwell in about 1954 contained the cream of the cream; what a hell of a risk that was". The best Japanese brains, meanwhile, were going into the engineering and manufacturing industries. "That's why they're knock-hell out of us now".

Thus Sir Ieuan finds himself "a bit of a renegade" on the Joint European Torus, because "if JET comes to Britain it's going to suck in another new generation of cream talent"—talent that is sorely needed in the engineering industries.

During his time at the three ministries, Sir Ieuan has worked with seven ministers: Cousins, Benn, Rippon, Davies, Walker, Benn again and Varley. His most stimulating time was at Mintech, working with Benn as minister and the formidable Sir Richard (Otto) Clarke as Permanent Secretary.

He quotes two main overall conclusions. First, the tragedy of 1970 when promising Mintech projects were killed off indiscriminately; clearly this still hurts. Secondly, the fact that all the various micro-economic attacks on particular industrial problems will be of little benefit without a stable, healthy macroeconomic background. "Unless you've got that", he says, "technology will not flow".

USA

From 63 to 400?

Colin Norman in Washington examines the implications for nuclear power of last week's energy proposals from President Carter

"THIS has been an exceptional week", President Carter declared last Friday during a packed press conference. Indeed it has. It began with a sombre televised address in which Mr Carter suggested that overcoming the energy "problem" would be a little like waging a war, and ended with a press conference in which he defended his strategy for winning that war. The strategy itself was unveiled on Wednesday, and it has so far achieved the distinction of uniting commentators in their observations that dramatic measures may be needed, but that some of Carter's measures should be resisted.

The strategy consists of a bold set of measures designed to curb Americans' seemingly insatiable appetite for energy by raising energy prices, taxing heavy users, and returning the revenues back into the economy in the form of rewards to people who conserve (see box). The plan is also designed to decouple industry from its reliance on oil and gas by forcing more widespread use of coal, and it also attempts to encourage the use of light water reactors. The emphasis is on the near term.

The proposals for nuclear power are particularly interesting. On April 7, Carter announced that, in the interests of curbing nuclear proliferation, the Administration will defer indefinitely its plans to reprocess commercial nuclear fuel and that it will sharply downgrade the breeder reactor programme. Last week, those decisions were spelled out in more detail, but at the same time, Carter made it clear that the Administration does not intend to turn its back on nuclear power. Far from it. Carter in fact announced that the Administration will seek to remove some of the roadblocks to licensing nuclear plants, step up attempts to find suitable burial sites for nuclear wastes, and completely change direction on uranium enrichment by building a large new enrichment plant using gas centrifuge technology instead of the present gaseous diffusion process.

The intention, in short, is to rely on light water reactors, without plutonium recycle, to meet a growing share of energy needs for at least the next 25 years, but to keep the breeder reactor effort ticking over as insurance against the possibility that the United States will need to move into the plutonium

economy early in the next century. According to officials of the Energy Research and Development Administration (ERDA), the number of light water reactors in the United States is expected to grow steadily from the present figure of 63 to between 300 and 400 by the turn of the century.

The nuclear strategy rests on some critical assumptions, however, perhaps the most important of which involves the size of the United States' uranium reserves. Proven reserves now amount to about 1.8 million tons, which would be sufficient to fuel about 300 reactors throughout their lifetimes—barely enough to meet ERDA's projections for domestic nuclear expansion this century, with none left over to meet contracts from abroad. ERDA is, however, launching an intensive programme to assess uranium reserves more accurately, and acting ERDA Administrator Robert Fri said last week that he is "guardedly optimistic" that proven reserves will swell to about 3.7 million tons. There is some question, however, whether the electricity in-

dustry, faced with uncertainties in long-term uranium supply and with no guarantee that plutonium recycle will be available to help stretch nuclear fuel supplies, will be enthusiastic about purchasing nuclear plants.

As for the breeder reactor programme, there is still some confusion about the Administration's intentions. One thing is certain, however: the effort is being sharply cut back and it no longer occupies pride of place as the top priority programme in the United States' energy research and development effort. Last week, Carter proposed that the programme's budget be reduced by \$173 million next year, in addition to a cut of nearly \$200 million which he proposed in February. The plan now is to complete the fast flux test facility, a test reactor now under construction in Washington, but to defer indefinitely plans to build a large demonstration reactor on the Clinch River in Tennessee. That project, which was to have cost more than \$2,000 million, will be redirected to focus on alternative breeder reactor designs which present less of a proliferation hazard. The exact nature of the programme has not yet been

The central feature of President Carter's energy strategy is a carrot-and-stick arrangement of rewards and penalties designed to break the country's addiction to vast quantities of energy. The carrot comes in the form of an assortment of tax breaks and other financial incentives for people who insulate their homes, buy small cars or install solar heaters. The stick is a complex set of proposals which will lead to higher energy prices, and the threat of stiff taxes on gasoline and on gas-guzzling cars. The goal is to reduce the growth in energy demand from its present level of about 3.5% a year to about 2%.

Coupled with those efforts to stimulate conservation is a series of proposals designed to increase the use of coal and light water reactors. Longer-term energy supply options will also receive modest increases in research support, but the emphasis is mostly on efforts likely to produce relatively quick results.

The major elements of the plan:

Oil and gas prices: A complex set of taxes coupled with price increases for newly-discovered domestic crude oil would cause oil and gas prices to increase until they "generally reflect the true replacement cost of energy". At present, prices are kept artificially low by a variety of controls.

Gasoline taxes: Unless gasoline consumption is reduced according to specific targets, tax increases of 5 cents per gallon will be imposed each year, beginning in 1979, until the total tax reaches a maximum of 50 cents.

Gas guzzler taxes: New cars which fail to meet gasoline consumption standards would be heavily taxed, and the revenues would be returned in the form of rebates to people who buy more efficient cars.

For example, 1978 model cars which get less than 12.5 miles per gallon would carry a tax of \$449, while those which get more than 39 miles per gallon would be given a rebate of \$473. By 1985, cars with those consumption levels would carry taxes and rebates of \$2,488 and \$493 respectively.

Insulation and Solar heating: All new buildings will have to meet insulation standards. Homeowners who insulate their houses would be entitled to income tax credits of 25% of the first \$800 and 14% of the next \$1,400 spent on conservation measures. Similarly, people who install solar equipment before 1985 would be able to claim 40% of the first \$1,000 and 25% of the next \$6,400 as a tax credit.

Nuclear power: Light water reactors without plutonium recycle will have an increasingly large role. New gas centrifuge plants will be built to enrich uranium for reactor fuels, more funds will be pumped into efforts to find suitable waste disposal sites, and an attempt will be made to reduce the present 10-year licensing process for nuclear plants to 3 years.

Coal: Many industrial facilities would be forced to switch from oil and gas to coal, and they would also be required to use the best available technology to control pollutants. Research and development on processes designed to produce synthetic oil and gas from coal would be stepped up. In addition, Carter will establish a commission to report by October this year on the health effects associated with mining and burning large quantities of coal. And a new research programme, costing \$3 million, will be launched to look into the so-called greenhouse effect.

worked out, however, and Administration officials suggest that it will be weeks, if not months, before it is.

As for the plan to build a large centrifuge enrichment plant, this again is coupled with Carter's moves to curb nuclear proliferation. In 1974, the United States closed its order books for long-term supplies of enriched uranium to other countries because of projections that US enrichment capacity would be used up. The move is said to have prompted many countries to seek alternative sources of supply, and it may have been a key factor in persuading Brazil to negotiate the purchase of an enrichment plant from West Germany. Carter has therefore announced that the United States will re-open its order books, and that a supply of enriched uranium will be guaranteed to those countries which renounce the development of nuclear weapons.

To meet the projected demand, a large centrifuge enrichment plant will be constructed at a cost of some \$4,400 million. So far, all US enrichment has used the gaseous diffusion process, and previous plans called for expansion of an existing diffusion plant at Portsmouth, Ohio. Administration officials say, however, that the gas centrifuge process, which has been developed in secrecy at Oak Ridge, Tennessee, is now ready to be commercialised. So far, however, no site for the new plant has been chosen, though hints that it may go to Oak Ridge, to compensate for cancellation of the breeder reactor project there, have sparked loud protests in Portsmouth.

In addition to relying on a modest expansion of nuclear generating capacity, Carter's plans for medium-term energy supply also envisage considerable expansion in the use of coal. He has proposed measures to force industry to convert from oil and gas to coal, announced large increases in research and development efforts aimed at producing synthetic oil and gas from coal, and recommended a number of regulations to reduce the environmental consequences of such an expansion.

There is, however, one potential environmental problem which could prove intractable: the so-called greenhouse effect which may lead to changes in the global climate from large increases in atmospheric carbon dioxide. Carter announced last week that a major study of the greenhouse effect, costing \$3 million, will be launched this year.

Carter's energy plan will now be picked over on Capital Hill, and already special interest groups are clawing at it. The debate is likely to rage for the rest of this year. □

GEOS

Hope from despair?

Stuart Sharrock in Washington and Chris Sherwell in London report on the ill-starred launch of Geos, the European Space Agency's scientific satellite

ON Tuesday of this week a group of space scientists and another of space engineers met separately on opposite sides of the Earth to discuss an event that occurred above the globe halfway between them. Last Wednesday, twenty minutes after a Thor-Delta 2914 rocket had taken off from Cape Canaveral only 0.2 seconds later than its scheduled lift-off of 05.15, the hopes of 11 research institutes from eight member countries of the European Space Agency turned to despair as a malfunction during the separation of the second and third stages of the rocket caused the third stage to separate early. It meant that the payload, the world's first geostationary scientific satellite known as Geos, could not achieve its planned orbit and that seven experiments which took years to prepare might no longer be done.

In fact the despair looks as though it may have been premature. For although the engineers from the rocket manufacturers McDonnell Douglas gathered in California to discuss the Delta's acknowledged failure, the scientists who met at the ESA's Space Operations Centre at Darmstadt in West Germany did so a little more optimistically. On the previous day Geos had been placed in an elliptical orbit whose apogee, at 38,498 km, was easily as high as the originally intended circular orbit.

The difference, of course, was that the new orbit, not being geostationary, would not allow the satellite to be permanently within sight of the Darmstadt ground station. It is understood, however, that not all the data obtained over each 24-hour period would have been used anyway. The new elliptical orbit, selected to maximise the return of scientific data, has a perigee of 2,131 km and an inclination of 26.85°. It is traversed every 12.06 hours by the satellite, which could now be within sight for some 10 hours a day.

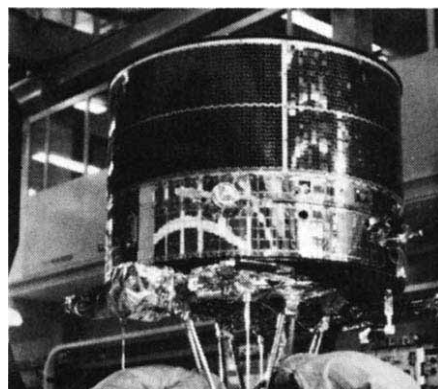
As the scientists gathered to decide which experiments to pursue and data to collect, ESA officials were pleased to have retrieved, if only partially, a mission which at first looked on the very edge of disaster. The spacecraft and third stage assembly should have spun up to 97 revolutions per minute before separating from the second

stage. This spin stabilisation would have ensured that the third stage had an accurate trajectory during its burn; without it, the spacecraft tumbled around into the wrong orbit, though it was stabilised soon thereafter.

The spacecraft could not have been allowed to stay indefinitely in this so-called transfer orbit because it went through zones of high radiation which might endanger the experiments and decrease the total active lifetime of the spacecraft. To stay in that orbit also served no useful purpose: it was not on the magnetic field lines connected to the auroral zones of the Arctic and Antarctic, and the experiments would probably not work with the field and particle intensities so high.

The original intention was that the spacecraft should be injected into an elliptical transfer orbit (apogee 35,500 km, perigee 230 km) and then be boosted on the fourth apogee to a circular orbit synchronised with the Earth below. That the European-built solid-propellant apogee booster motor responsible for this task placed Geos in the elliptical orbit it now occupies is a cause for comfort, even though the motor could not be used twice to achieve a circular orbit. But then so much could so easily have gone wrong.

The effect, ironically, is that Geos is now placed in a fashion that had been expected of the ESA's spare 'qualification' geosynchronous satellite which was due to be sent up in 1979 atop a European Ariane launcher now being developed. It remains possible that this back-up satellite will be upgraded to become the 'true Geos', but it will cost the ESA another 13 million units of account to purchase the launch from NASA—a cost which is not recoverable for last week's launch under NASA's no-loss-no-profit contracts. Although the ESA Council is due to meet next week, it is unlikely to discuss this idea of inverting the roles of the two satellites. □



Quarks out of the bag?

In a paper given at the American Physical Society Spring Meeting in Washington DC, Drs W. M. Fairbank, A. F. Hebard and G. S. LaRue of Stanford have presented evidence for the existence of fractional electric charges. The experimenters emphasise that they are claiming the existence of fractional charge; they do not mention quarks.

Their experiment is a sophisticated version of the classic Millikan oil-drop apparatus. A superconducting niobium sphere 0.25 mm in diameter is floated in a magnetic field and its net residual charge deduced by analysing its behaviour in an oscillating electric field, the net charge can be altered by integer values of the electron charge e , by using a radioactive source. Fractional charges of $\frac{1}{3}e$ or $\frac{2}{3}e$ carried by a quark would be detected as the sensitivity claimed is 1% of the electron charge.

The most severe background is electric and magnetic dipole moments on the spheres which mimic the effect of a fractional charge. These dipole backgrounds are now understood and have been eliminated, according to the experimenters.

Of eight spheres measured, two show evidence of fractional charge: the first was seen last September and the second in mid-February. One had a measured residual charge of minus $0.331 \pm 0.070 e$ which went to zero after handling at room temperature. A second sphere has a charge of plus $0.337 \pm 0.009 e$. It has been measured on three occasions and the charge remained after the sphere was taken to room temperature.

Preparation of the spheres includes heat treatment during which five spheres were supported by niobium and three by a tungsten substrate. The two spheres showing fractional charges came from the tungsten substrate set.

Why have all other quark searches been fruitless? In an interview with *Nature*, Dr Fairbank argued that searches have only been carried out on a limited variety of materials. If quarks were released in extremely energetic events during the early stages of the universe one can speculate that they go through some unknown evolutionary process to end only on heavy elements.

Initial reaction to the announcement is firstly surprise that quarks could be so abundant as to show up on two out of only eight spheres, and secondly amazement that an antiquark is seen. The experiment cannot distinguish a residual charge of $+\frac{1}{3}e$ from $-\frac{2}{3}e$.

If these quarks are genuine they will challenge much recent theoretical work. Present understanding of quarks inside protons is that they are light and bound within the proton—to remove one would require an infinite amount of work. Heavy free quarks could be accommodated within the theory but they would be very different objects from those inside the proton and much of the aesthetic beauty of the theoretical framework would be lost.

Future plans of the Stanford team include measuring more spheres, investigating the significance of their mode of production and evaporating the material to separate and collect the charges.

Stuart Sharrock

IN BRIEF

Brenner's appointment

The UK Medical Research Council (MRC) has announced that Dr Sidney Brenner will become leader of the MRC Laboratory of Molecular Biology (Hills Road) in Cambridge on the retirement of Dr Max Perutz in 1979.

The news is little surprise for those who have heeded the rumours that followed an internal MRC report produced last year on the future of Hills Road, suggesting a radical change in organisation. The MRC has followed this, for whereas Dr Perutz was chairman of the Governing Board of the laboratory, Dr Brenner is to be its Director.

The Governing Board at present consists of Dr Perutz, Dr Hugh Huxley (whose appointment as Deputy Chairman has also been announced), Dr Brenner, Dr John Gurdon, Dr Cesar Milstein, Dr Fred Sanger and Dr John Smith. It has been collectively responsible for the running of Hills Road, with no particular power being vested in the chairman.

In 1979, however, the Governing Board will probably be disbanded. Its replacement, if any, is currently under discussion, but whatever the outcome Dr Brenner is certain to end up with a good deal of power. What is more, several of the senior staff at Hills Road will have left by the time Dr Brenner becomes Director. Dr Francis Crick has recently announced that he is to remain at the Salk Institute where he is currently on sabbatical, and David Blow has just taken up a chair at Imperial College, London. It is also rumoured that two other senior members, both on the Governing Board, have their eyes on other posts.

Another novelty for Hills Road is the fact that it is a shrinking laboratory. The staff is to be gradually pruned by 25%, back to its 1972 level.

All told Sidney Brenner will have a considerable challenge on his hands if he is to maintain the world-wide reputation of Hills Road during the course of his Directorship, which automatically lasts (as does the laboratory) until 1987, when he is 60 years old. Dr Brenner was recently heard to say "I am going downhill and I am going with a lot of noise". Only the second part of that statement is true.

Atkinson for NSF

Richard Atkinson, a former professor of psychology at Stanford University, has, as expected, been named by President Carter to be Director of the US National Science Foundation (NSF). Generally regarded as an effective administrator well versed in dealing

with Congress, Atkinson has been Acting Director of NSF since August 1976, when the previous incumbent, H. Guyford Stever, departed for a brief stint as science adviser to President Ford. Atkinson was deputy NSF director for 17 months before that. A top-flight researcher in fields ranging from the development of memory to the application of computers to instruction, Atkinson is the first social scientist to be appointed Director of NSF.

Whose power to choose?

There is now "a unique and possibly unrepeatable opportunity" to bring international action to redirect the worldwide use of nuclear power, according to Brian Johnson, author of a paper published by the International Institute for Environment and Development, *Whose Power to Choose?* At a time when President Carter is committed to controlling nuclear proliferation and the US nuclear industry gets 70% of all nuclear power station orders, there should be the opportunity, he argues, to bypass the plutonium economy by assuring countries of fuel for the thermal reactors they need, safeguarding the international community from proliferation and creating programmes to develop alternative energy sources.

To implement such proposals he suggests a reformed IAEA: its Department of Safeguards and Inspection should be strengthened, all nuclear facilities should be licenced with the IAEA to ensure security and environmental safety, and it should enforce internationally agreed measures for waste disposal. A new energy agency could be developed, independent of the United Nations but with support from OPEC and the US, to look into ways of helping poor countries shed their dependence on oil and nuclear power by developing alternative energy sources.

Uranium for Europe

President Carter's statement in last week's energy message to the US Congress that it was time "to resume uranium enrichment services" to Europe and elsewhere is likely to be well received in the European Community. The Nine's dependence on imported energy and on nuclear power was stressed heavily by the President of the European Commission, Mr Roy Jenkins, when he met Mr Carter earlier in Washington. Mr Jenkins told Mr Carter that without adequate and assured supplies of enriched uranium, European countries would be more

tempted to invest in reprocessing and fast breeders.

Supplies from the United States were held up pending a review of US policy on nuclear exports: a total of almost 2,900 pounds of uranium, for various destinations around the world, was involved. Canada, Europe's other main supplier, has held up supplies too, pending full agreement on safeguards and nuclear exports.

Preparing for 1979

The ten members of the Committee on Science and Technology in Developing Countries (COSTED)—a grouping established within ICSU in 1966—gathered in London for a couple of days last week for their annual meeting. Among other things, they are understood to have discussed their position in relation to the forthcoming United Nations Conference on Science, Technology and Development, taking the view that a non-governmental group of scientists ought to participate in their individual capacities. COSTED also

hopes to help in the preparation of papers by developing countries.

UK vacancies

During 1976 the British Civil Service had vacancies for about 340 scientific officers at grades up to Senior Principal Scientific Officer. About 11,000 applications were received, but even so only 250 appointments were made, the Civil Service Commission says in its annual report. It seems that recruitment of scientists is a particularly tricky business; as many as 50% of those made offers decline to take them up.

Public expenditure cuts obliged a number of major departments to withdraw entirely from recruitment in the second half of the year.

Concorde's noise

A gloomy report on Concorde noise levels has emerged from a working group of the Noise Advisory Council (HMSO, 50p). So gloomy in fact that the Secretary of State for the Environment, who is chairman of the council,

specifically states that the views expressed are those of members of the council only and not of the Secretary of State.

The first operational figures for Concorde noise at Heathrow were obtained in the summer of 1975. They showed take-off noise to be on average several dB above Heathrow's statutory day-time limit of 110 PNdB at certain monitoring points. The working group were told that an improvement of a few dB could be expected in cooler weather and when pilots became more experienced.

Operational figures for eight months of commercial operations are now available. The working group consider there has been little or no improvement on the 1975 figures, and suggest that the only way to improve Heathrow's noise climate would be to ban Concorde at night and in the evening and early morning, to restrict other jet flights at night still further, and to require Concorde operation to balance its increased noise with reductions in flights of other noisy aircraft.

DURING the past ten years many writers have suggested that global pollution is the most serious hazard facing mankind. They say that if we insist on economic growth and further industrialisation and on using more energy, catastrophic environmental degradation is inevitable. This was the message of *The Limits to Growth* and *A Blueprint for Survival*. It would be foolish to deny that pollution is a problem, or that new industries may give rise to serious damage. We should welcome attempts to regulate developments such as those set out in "Suggestions for the development of a hazard evaluation procedure for potentially toxic chemicals", a memorandum from the Monitoring and Assessment Research Centre (MARC) at Chelsea College, London. But we should also recognise that many new techniques are much less polluting than those which they replace, and that any alleged correlation between the use of energy, or a rise in GNP, with pollution, is spurious.

In fact, changes are often in the other direction. London's air is cleaner in 1977 than it was in 1877, though the population has increased and far more energy is consumed. In their recent book, *The Next 200 Years*, Herman Kahn and his colleagues of the Hudson Institute suggest that vastly increased economic growth and energy consumption will be needed to ensure global cleanliness. The same view is, in effect, held by

the British government. Thus drastic measures are set out in the Control of Pollution Act passed by Parliament in 1974 which was intended to activate the law to reduce all types of environmental pollution. However,

Pollution economics



KENNETH MELLANBY

many of the most important sections of the Act have not yet been brought into operation.

It is generally agreed that, in Britain as in most industrialised countries, river pollution is the most serious problem. We know how our fresh waters could be improved, and we have the methods to do so. Yet it is just those provisions which would

most affect our rivers which are in abeyance, and this is because our economic record is so dismal and our growth so slow. Mr Denis Howell, on behalf of the Secretary of State for the Environment, confirmed this in *Hansard* on 17 February, when he informed Parliament that "Implementation of the remaining provisions [of the 1974 Act] has been delayed because of present constraints on public expenditure but will take place as soon as possible".

This lack of legislative progress is disappointing, but is it important? All the evidence suggests that, during recent years, our dirtiest rivers have continued to improve even without the force of the new law. Every year more species of fish return to the Thames, and we now have a report that there is a healthy population of three-spined sticklebacks in the River Clyde off Glasgow Green—the first instance of fish breeding in this area for over a hundred years. Even with their limited resources authorities with the will to succeed have found a way to improve the situation. To restore salmon and trout to the Thames and the Clyde will take time, and the expenditure of a great deal of money for more and better sewage treatment. Only industrial growth or a drastic reorganisation of our economic system can supply the funds. To try to enforce regulations for which money is not available is a recipe for chaos, not for a clean environment.

correspondence

Scottish science

SIR,—Martin Goldman's distaste for the idea of devolution in Scotland (17 March, page 208) should not let him mislead readers by saying that Scotland does well out of grant-giving bodies such as the Medical Research Council (MRC).

The MRC spends 8.6% of its budget in Scotland which has 9.7% of the UK population (*Hansard* 18 December 1975) and if the figure were based on the number of medical students or teaching staff it would be 17%.

I am told by those who advise the MRC that the reason the Scottish figure is low is that Scottish applications are rare, and that the reason for this is that the Scots believe that the clever people in London are more deserving of the money. The only cure for this affliction of the spirit is devolution or federalism.

Finally, Dr Goldman's view that devolution or federalism cannot work will be read with amusement in North America.

Yours faithfully,

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Nuclear irrationality

SIR,—My brief essay "Nuclear Power, Public Interest and the Profesional"¹ was essentially a plea for critical rationality (and the consequent anti-authoritarian approach) in the way society evaluates nuclear power. Two letters^{2,3}, present critical comments which I think are partly irrational.

Dr Grantham makes several misleading statements. One example is the comment on small reactors and "bomb-capacity commercial reactors" in relation to "a few microgrammes (of plutonium) can kill you". Another example is "the (nuclear) battle is one-sided. Pronuclear propagandists are paid: the opposition is not". Apart from pointing to the falsehood in the latter part of this statement, I emphasise that I do not look at the nuclear debate as a battle. My essay was an attempt to get at the facts and to emphasise the need for a rational debate on all "sides".

Dr Grantham has also chosen to

disregard completely almost all my basic points. He states that "nuclear industry representatives seem to want to exclude biologists, environmentalists and the general public from the controversy", and "instead of trying to restrict the discussion to experts we should indeed extend it to everyone". In this connection I refer to two of my statements: "Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad participation", and "The scale and timing of the introduction of nuclear power is a major and complex decision. In a democratic society such decisions are not left to technological experts but to elected political officials".

Dr Grantham objects to what he calls my "suggested dichotomy: reasonable, rational and valid" versus "irrational, emotional and ethical". Again a quote from my essay is relevant: "Clearly, a number of irrational, emotional and ethical factors may be and should be of importance in a choice of power plants for the production of electricity".

On the role of scientists in public debate, Dr Anfinen states that "anyone but the most naive petition-signer must understand that sponsors of socio-scientific news releases are, and always will be, selected on the basis of the fact that the general public tend to be influenced by lists of the names of 'dignitaries'". This is precisely the basis for my criticism of petition signers using their titles when these are irrelevant to the matter. For example, a responsible nuclear physics professor will know if his personal background justifies him speaking on the nuclear energy question as a nuclear physicist (with a consequent intended and justified substantial impact on politicians and the public), or if he ought to speak as Mr J. Q. Public. Hence my plea that specialists should not mislead the public by carrying their specialist background as a flag in unrelated issues.

Incidentally, in referring to E. Rabinowitch (for whom I have great respect) as "a person concerning whose judgement and expertise in the area of science and public policy no one would quibble", Dr Anfinen appears to embrace precisely the authoritarian approach which parts of my essay were directed against.

For Dr Anfinen, as for myself, the

question of a connection between nuclear power and potential nuclear wars is a key issue. It deserves not only the religious and emotional considerations put forward by Dr Anfinen and the National Council of Churches of Christ in the USA, but also our utmost rational efforts to reduce the risk of such wars, with or without nuclear power. I fail to understand the rationality of distracting public attention from the all important issue of nuclear disarmament by expending substantial efforts on stopping the peaceful uses of nuclear power, which probably decrease rather than increase the risk of war.

Finally, I offer, upon Dr Grantham's request, my definition of rationality: to be rational and reasonable is to apply flexibly the control of our knowledge and our theories to our actions, so that we can perform the continuous trial and error process required for improving our adaptation to the environment by way of critical argumentation, thus reducing the need to subject our physical beings to those vagaries of nature and emotions which are harmful.

The place of facts in the world of human values should be set by the values, but facts cannot be changed by values.

Yours faithfully,

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¹ Doderlein, J. M., *Nature* 264, 202 (1976)

² Oranham, R., *Nature* 265, 100 (1977)

³ Anfinen, C. B., *Nature* 265, 492 (1977)

Requests for reprints

SIR,—Would you publish an appeal to scientists throughout the world on a matter which is on the one hand extremely trivial but on the other could eliminate a nuisance. I refer to the request for reprints by fellow scientists. Besides the cost of mailing these reprints, it is time consuming to cut out addresses from post cards, glue them onto envelopes, decipher poorly written names and very often have to write the whole address by hand or typewriter. It would make life so much easier if requests in future carried a stick-on label as a few from considerate colleagues already do.

Yours faithfully,

F. ECKSTEIN

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news and views

Formation of planetary atmospheres

from Robert Hutchinson

THE inner planets are usually considered as having formed by the accretion of solids at least part of which could release a volatile fraction when heated. Outgassing from hot interiors would then have led to the formation of atmospheres. To understand the development and evolution of a planet's atmosphere therefore, the bulk chemical composition of the primitive material from which the body was built must be established. This is not an easy task; the bulk composition of the best known planet, Earth, is still disputed today.

During the past 10 years, cosmochemists, largely led by Anders' group at Chicago, have found that many meteorites are composed of fractions each representing stability at a particular temperature/pressure condition. Heterogeneity in meteorites led to the idea of heterogeneous accretion of planets (Harris & Tozer *Nature* **215**, 1449; 1967; Turekian & Clark *Earth planet. Sci. Lett.* **6**, 346; 1969).

Essentially, the Earth is thought to have formed by growth of a small body composed of refractory Fe-Ni metal and/or Ca-Al-Ti-rich oxides and silicates. As it grew, lower temperature Mg-Fe silicates were added until, lastly, accretion ceased with the addition of a hydrated, low temperature veneer. Heating of the veneer released water for our oceans and gases for our primitive atmosphere. Like many theories in planetary science, heterogeneous accretion is not without its critics. And the

largely qualitative treatments of the 1960s are now being quantified.

It is against this background that the paper by Rasool and Le Sergeant in this issue of *Nature* should be viewed (page 822). The Viking landers obtained an estimate of the chemical composition of the martian atmosphere, so there is now reliable information on the atmospheres of two inner planets which must be accommodated in any model of atmospheric development. Using the simplest treatment, Rasool and Le Sergeant assume the terrestrial and martian atmospheres to be essentially closed systems. They begin by looking at the better known Earth and attempting to find a suitable source material for the volatiles. Carbon- and water-rich carbonaceous chondrite meteorite is rejected because the Earth's atmosphere has too much ^{20}Ne and other heavier inert gases relative to C (and H). Instead, the volatile-poor LL group of ordinary chondrites is chosen as it gives a better match, especially for the inert gases. It is argued that a 116-km thick veneer of LL group chondrite could have been outgassed to produce oceans and atmosphere.

When a similar model is applied to Mars it is found that the quantity of LL group chondrite required to produce the atmospheric ^{36}Ar content would produce more N, C and H than are observed in the atmosphere. The amounts of ^{84}Kr and ^{132}Xe agree with the ^{36}Ar abundance. Rasool and Le Sergeant go on to argue that if

the N, C and H are not in the atmosphere, they may be in the regolith or on the martian surface; for example, H may be present as polar water ice up to 40-m thick. Near surface volatiles could be temporarily released to form an atmosphere of CO_2 and H_2O with surface pressure of 400 mbar.

Finally, the high ^{40}Ar content of the martian atmosphere is considered in the light of the low upper limit (0.25%) for K in the martian surface (^{40}K is the radioactive parent of ^{40}Ar). Because the martian surface is apparently not as enriched in K as is terrestrial continental crust (but compare oceanic tholeiitic basalt, with less than 0.1% K) Rasool and Le Sergeant argue that ^{40}Ar on Mars has come from a greater thickness of material than the other volatiles. It is also implied that K has not been concentrated upwards on Mars due to lack of complete melting in this planet.

It is likely that Rasool and Le Sergeant's paper will prove to be controversial. A probable line of attack is that terrestrial O isotopic ratios define a chemical fractionation line enriched in ^{16}O relative to LL group (and other) ordinary chondrites (Clayton *et al.* *Earth planet. Sci. Lett.* **30**, 10; 1976). This argues against an LL group origin for terrestrial volatiles, but for the foreseeable future, martian O isotopic ratios will remain unknown. $\square$

Redox potentials of iron-sulphur clusters

from A. J. Thomson

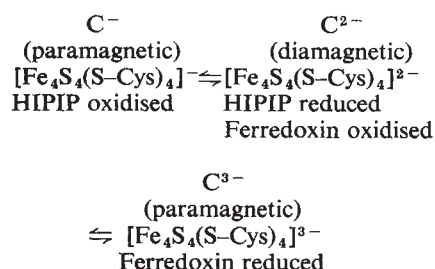
THE non-haem iron proteins consisting of clusters of iron and sulphur are known to be involved in a wide range of biological processes from steroid hydroxylation and CO_2 and N_2 fixation to oxidative phosphorylation and photosynthesis. They have been isolated from obligate anaerobic bacteria, aerobic bacteria, algae, plants and all animals so far investigated. Their single most important function is the catalysis of oxidation/reduction reactions and

the transport of electrons to reaction centres.

For this reason the range of redox potentials exhibited by the Fe-S clusters is of great interest. It is now emerging that proteins containing a common structural Fe-S centre can have widely differing redox potentials. For example the high potential iron protein (HIP) from *Chromatium vinosum* and the bacterial ferredoxin from *Peptococcus aerogenes* are close structural relatives.

The former contains a single cubane-type four-iron sulphur cluster $[\text{Fe}_4(\text{S-Cys})_4]$ whereas the latter possesses a pair of these units. However, functionally they are very different. HIP in the oxidised form gives an EPR signal which is abolished on reduction, and the mid-point potential of this process is +340 mV. By comparison the bacterial ferredoxin exhibits a one-electron reduction also but to give an EPR signal in the reduced state only and with a mid-point potential

of about -400 mV. These puzzling observations were neatly explained by a hypothesis due to Carter *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **69**, 3526; 1972) which suggested that the clusters in the two proteins were undergoing reversible redox changes between two different oxidation states, in the following manner



It is not therefore to be expected that the two proteins will show similar mid-point potentials or EPR characteristics. The three-state hypothesis was quickly given confirmation by Cammack (*Biochem. biophys. Res. Commun.* **54**, 548; 1973) who showed that HIPIP could be further reduced by dithionite to the C^{3-} state termed the super-reduced form of HIPIP in the presence of more than 70% by volume of dimethylsulphoxide which serves to unfold the tertiary structure of the protein. This demonstrated that the protein environment prevents further reduction or oxidation of the cluster, ensuring that only single electron transfers are carried out by the protein. Subsequently it was shown that in the presence of ferricyanide the bacterial ferredoxins can be super-oxidised to produce an EPR signal similar to that of oxidised HIPIP (Sweeney *et al. Biochem. biophys. Res. Commun.* **59**, 188; 1974).

Although crystal structures are available for both HIPIP and the bacterial ferredoxin from *Peptococcus aerogenes* it is not clear what features of the protein environment control the redox potential of the cluster. In the past eighteen months two new examples of iron-sulphur proteins have been investigated which show that the control by the protein environment is remarkable. The first example is a protein from *Azotobacter vinelandii*, FdI, which has been obtained in crystalline form and shown to contain two four-iron clusters. (Sweeney *et al. J. biol. Chem.* **250**, 7842; 1975). As isolated the protein shows an EPR signal very similar to that from super-oxidised bacterial ferredoxins. The signal disappears on photoreduction with spinach chloroplast fragments with a mid-point potential of -420 mV. Apparently the cluster is undergoing the C^- to C^{2-} reduction with a mid-point potential ~ 760 mV below that of the HIPIP cluster. Furthermore, oxidation of FdI with ferricyanide produces an EPR signal on uptake of one electron but with a mid-point potential of +350 mV. It is concluded that *A. vinelandii* FdI is the first example of an iron-

sulphur protein which contains two clusters functioning between the same pair of oxidation states as HIPIP but with mid-point potentials 760 mV apart.

The second, and more recent, example is seen in three ferredoxins (FdI, FdI' and FdII) extracted from the sulphate reducing bacterium, *Desulphovibrio gigas* (Cammack *et al. Biochim. biophys. Acta* **490**, 311; 1977). With molecular weights of 18,000, 18,000 and 24,000, respectively, the three proteins appear to be oligomers of a basic unit of molecular weight 6,000 containing one four-iron cluster. By monitoring the appearance and disappearance of the C^- and C^{3-} states with low temperature EPR measurements the mid-point potentials of clusters within the three proteins have been determined. Their values are summarised below.

	FdI	FdI'	FdII
C^-	$\updownarrow$	$\updownarrow$	$\updownarrow$
	-50 mV	-30 mV	-130 mV
C^{2-}	$\updownarrow$	$\updownarrow$	$\updownarrow$
	-455 mV	-130 mV	-437 mV
C^{3-}	$\updownarrow$	$\updownarrow$	$\updownarrow$

The three ferredoxins differ markedly in the intensities of the various EPR signals leading to the conclusion that FdI undergoes reduction between C^{2-} and C^{3-} states, FdII between C^- and C^{2-} states and FdI' can be produced in both C^- and C^{3-} states. There is no evidence that a single cluster can reversibly change between all three oxidation states. A more plausible interpretation is thought to be that in some subunits of the protein the four-iron centre undergoes the C^-/C^{2-} transition whereas in other subunits of the same protein the $\text{C}^{2-}/\text{C}^{3-}$ transition can take place.

Although the three-state hypothesis undoubtedly correctly assigns the oxidation states to the clusters, the very property it sought to explain—the difference between the redox potentials of HIPIP and bacterial ferredoxins—requires more subtle interpretations in the light of these new results. Several mechanisms for the control of the cluster redox state by the polypeptide have been discussed. It has been shown that the cluster geometries in oxidised and reduced *Chromatium* HIPIP are significantly different. This led to the suggestion that the size and shape of the cluster-binding cavity might be designed to favour one oxidation state. (Carter *et al. J. biol. Chem.* **249**, 6339; 1974). Alternatively, a given oxidation state could be stabilised appreciably by the formation of hydrogen bonds between the cluster and the protein. Carter and coworkers (*op. cit.*) have identified $\text{NH} \cdots \text{S}$ hydrogen bonds in their crystal structure determination of HIPIP. Finally,

it may be significant that the newly discovered examples are all of proteins containing more than one cluster. It could be, as Sweeney, Rabinowitz and Yoch suggest, that inter-cluster interaction is strong enough to modify mid-point potentials. The study of water-soluble model compounds could provide some interesting comparative data.

New light on old mesons

from Peter J. Litchfield

THE excitement generated in the high energy physics community by the discovery of the ψ/J mesons and the emergence of the 'new physics' of charm has tended to overshadow developments in the field of conventional meson spectroscopy. However, new results from a partial wave analysis of the reaction $p\bar{p} \rightarrow \pi^+\pi^-$, just published by a Queen Mary College, Daresbury Laboratory and Rutherford collaboration (*Phys. Lett.* **67B**, 117; 1977), have shown that the old-fashioned charmless physics still has some surprises in store. The groups have determined the spins and parities of three resonances with masses approaching those of the ψ mesons; by some way the heaviest conventional mesons with well-established quantum numbers. The states seem to lie on a daughter Regge trajectory with unexpected properties which, if confirmed, could cause a major upset in our theoretical ideas. The minimum disruption would require a modification of the predictions of Regge models at high energies and momentum transfers. It could, on the other hand, be possible that these resonances are manifestations of four quark 'exotic' states and signal the opening of a whole new branch of particle physics.

The search for high mass resonances is complicated by their small branching fractions into any given final state and their large widths which result in strong overlapping of adjacent resonances. The only satisfactory experimental access to them is by way of formation experiments in which measurements of the differential cross sections and polarisation parameters are made as a function of centre-of-mass energy. This enables the reaction to be decomposed into its constituent partial waves and thus enables resonances in a given spin parity (J^P) state to be observed. The new discoveries have been made possible by high statistics measurements of the differential cross sections and polarisations for the reaction $p\bar{p} \rightarrow \pi^+\pi^-$ in

the centre-of-mass energy range 2,020 to 2,580 MeV made at CERN by the QMC, Daresbury and Rutherford groups (together with physicists from Liverpool for the differential cross section data). They are the first measurements which are sufficiently precise to enable a definitive partial wave analysis to be performed in $\bar{p}p$ annihilations. The analysis has revealed the presence of resonances at masses of 2,150, 2,310 and 2,480 MeV/ c^2 with spin parities 3^- , 4^+ and 5^- respectively. The two lower mass states have been observed before as bumps in the total and elastic cross sections but this is the first determination of their quantum numbers.

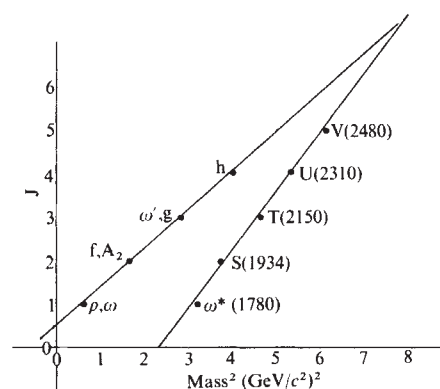
One method of classifying resonance states is to plot them on a graph of spin versus mass squared where states are found to lie on straight line trajectories known as Regge trajectories. Figure 1 shows this plot for the dominant mesons found in production experiments, the ρ , ω , f , A_2 . . . , together with the three new states, another pp resonance found at a lower mass (the S(1934)) and a 1^- state recently reported from an e^+e^- annihilation experiment at Orsay. There are clearly two separate trajectories, one for the dominant mesons in production and one for the dominant mesons formed in pp annihilation experiments.

The plot yields two surprises. First, the lower trajectory (called a daughter trajectory) is not parallel to the leading trajectory. Regge phenomenology has always assumed that trajectories are linear and parallel. Although very closely linear, the slope of the daughter trajectory is considerably greater than that of the leading trajectory. This could have important consequences if the trend continues since at higher energies and momentum transfers the new trajectory would dominate the ρ trajectory which is the only one usually retained in theoretical calculations.

Second, it is puzzling that the dominant resonances in $\bar{p}p \rightarrow \pi^+\pi^-$ are not on the leading trajectory as is the case in all other known reactions. An observation by this same group from their data on $\bar{p}p$ elastic scattering taken as a by-product of this experiment (*Nucl. Phys. B113*, 1; 1976), indicates a possible explanation. In that reaction they find that there is a characteristic maximum radius of interaction of approximately 1.5 fm. If this is also true in the $\pi^+\pi^-$ reaction and we assume a simple impact parameter model where the angular momentum is given by the radius of interaction times the centre-of-mass momentum, then the maximum angular momenta accessible at the resonance masses are those on the daughter trajectory. Resonances on the leading trajectory could not be formed.

An alternative but much more speculative explanation is that the new states are qualitatively different from those on the ρ trajectory, that is, they are made up of two quarks and two antiquarks rather than only one of each. Speculation on the existence of such exotic states has been rife in the theoretical world for several years and there has recently been rather weak evidence that such states may be formed in e^+e^- annihilations around 4.03 GeV (see *Nature* **266**, 301; 1977) and in $\bar{p}p$ annihilations at 12 GeV/ c (*Phys. Lett.* **66B**, 185; 1976). Theoretically pp formation experiments would be the ideal place to observe such states since an interaction in which a quark-antiquark pair was annihilated from the three quarks in the proton and three antiquarks in the antiproton would leave a state consisting of just two quarks and two antiquarks.

$\bar{p}p$ annihilations have long been advocated as the place to search for high mass mesons. It is only now after many years of effort that data of sufficient quality to realise these expectations have been obtained. Many questions remain. Does the new trajectory continue linearly and overtake the leading trajectory, or does it eventually flatten out? Data at higher masses



could give the answer. Better data on the K^+K^- channel (also measured in this experiment but with much lower statistics) or on the 3π channel would give access to states not available in the $\pi\pi$ channel. Do they have similar properties to those described here? Does the $\bar{p}$ just have a propensity to interact at a range of about 1.5 fm or are these states truly exotic? The interest of high energy physicists in conventional meson spectroscopy has been reawakened and already new experiments to investigate the $\bar{p}p$ system have been proposed. □

Picornavirus RNAs are interesting . . . and unique

from T. J. R. Harris

APART from satellite tobacco necrosis virus RNA, picornavirus RNA molecules provide the only well established exception to the 'capping' rule. These messenger RNA molecules lack the $m^7G(5')ppp(5')X$. . . that is characteristic of so many virus specific and cellular mRNAs from eukaryotic cells (see *Nature*, **263**, 188; 1976).

Experiments with polynucleotide kinase, an enzyme from T4-infected *E. coli* which adds ^{32}P from $\gamma^{32}P$ ATP to 5' OH groups, initially suggested that the 5' end of poliovirus RNA was pAp. The possible endonuclease contamination of the kinase preparation used made this experiment rather unsatisfactory and the discovery of a 5' cap on various virus mRNA molecules (particularly those of other animal viruses which have a genome that is a messenger RNA, Sindbis virus for example) led to a re-examination of the 5' terminus of poliovirus RNA, as well as that of other picornavirus RNAs.

Several papers have been published in the past year reporting the outcome of these new investigations. It is clear that the 5' terminus of 35S poliovirus-

specific mRNA isolated from the polyribosomes of infected cells is pUp. On the other hand neither a nucleoside diphosphate nor a capping group was found on the RNA extracted from virus particles. As the genome RNA presumably acts as a messenger RNA to initiate infection, this was a distinctly puzzling result. It is not surprising that an answer to the apparent paradox was soon forthcoming. Wimmer's group (Lee *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 59; 1977) and Baltimore and his co-workers (*Proc. natn. Acad. Sci. U.S.A.* **74**, 961; 1977) now have evidence that there is a small protein (molecular weight around 7,000) covalently linked to the 5'-nucleoside diphosphate. As well as explaining the difficulty of finding a 5' end, the presence of the protein on nascent RNA chains in the replicative intermediate has led both groups to speculate on a role for the protein in RNA synthesis, although some function in virus particle morphogenesis is not discounted.

Another interesting structural feature of picornavirus RNAs, unique among animal virus RNAs, is provided by the RNA of viruses belonging to the cardiovirus sub-group. Like some

plant virus RNAs such as tobacco mosaic virus (TMV) and turnip yellow mosaic virus (TYMV), cardiovirus RNAs can be aminoacylated *in vitro*; mengovirus RNA with histidine and encephalomyocarditis virus (EMCV) with serine. The part of the RNA molecule which is aminoacylated has not yet been determined. In contrast the RNA of poliovirus and foot and mouth disease virus (FMDV), both members of different picornavirus sub-groups, cannot be aminoacylated.

Although not containing a capped 5'-end, RNA molecules from representatives of all the picornavirus sub-groups possess the 3'-terminal poly(A) charac-

teristic of a number of other virus mRNAs. In addition to poly(A) the RNA of the cardioviruses contains a poly(rC) tract. Until recently the location of this homopolymeric region in the RNA was not known. Two papers published at the end of last year now indicate that the poly(C) is very near the 5'-end of both EMCV RNA and FMDV RNA and there is the possibility that the sequence is 5'-terminal.

It will be interesting to determine the structural basis of these differences in the RNAs of the various picornaviruses and to discover their functional significance in the replication of the viruses. □

Magnetostratigraphic pitfalls

from George Kukla and J. D. A. Zijdeveld

CONTROVERSY is growing over the existence of young excursions of the Earth's magnetic field such as the Gothenburg event (Morner & Lanser *Nature* **251**, 408; 1974). Are these features real? Are they true records of magnetic anomalies? Do they influence climate (Fairbridge *Nature* **265**, 431; 1977)? Or are they merely a product of overlooked natural or artificial strata deformations (Thompson & Berglund *Nature* **263**, 490; 1976; Opdyke *Earth planet. Sci. Lett.* **29**, 238; 1976)? The recently reported detailed study of Koobi Fora formation in Kenya (Hillhouse *et al.* *Nature* **265**, 411; 1976) shows that the magnetic reversal stratigraphy of Pre-Brunhes formation is not without difficulties either. With all these problems, can palaeomagnetic 'magic' still be taken seriously?

The participants* of the steering committee session of Project 128 of the International Geological Correlation Program concluded at a meeting in Paris, last September, that it can. The project, a joint venture of UNESCO and of the International Union of Geological Sciences was organised to foster intercorrelation of magnetostratigraphic zones with regional stratigraphic systems and radiometrically dated levels. It is a continuation of former IGCP Project PA.70.17 (see Kukla & Nakagawa *Geology* **3**, 704; 1975).

But there are complicating features to which increased attention should be paid. The most important of these are the presence of post-depositional magnetostratigraphic overprints, natural deformation of the strata under investigation, deformation caused by the coring and sampling devices and the continuity and sedimentation rates of the deposits.

Finding the remanent magnetisation of a sample is only one of three equally important procedures in magnetostratigraphy. The other two are—establishing the original position of the sample at the time of deposition, and the correlation of recognised magnetozones with the standard palaeomagnetic time scale.

Unchanged position of studied layers

The remanent vector of samples can only be related to the direction of the Earth's magnetic field if the original orientation of the layer is preserved or the amount of later deformation known. Most researchers assume that their oriented samples precisely represent the layer at the time of deposition and dedicate lamentably little effort to testing whether this is really the case. In studies of secular magnetic variation and of magnetic anomalies, this is a critical limiting factor. Unfortunately, the occurrence of local deformations due to slumping, crust loading, mudflows, and so on, even in generally well stratified horizontal beds is common. Such features can be detected in exposures (Verosub *Science* **190**, 48; 1975) but easily overlooked in cores. The coring operation in unconsolidated sediments further increases the incidence of unrecognised deformations. Arching, twisting, and 'sucking in' of sediment frequently affects the cores as can be observed on samples from laminated deposits. Also non-vertical penetration of the corer and systematic inclination errors affect the reliability of data. It is difficult to detect a deformation of homogeneous unstratified layers, although X-ray screening may prove helpful.

Plastic deformations may frequently occur at lithologic boundaries such as

sand-clay interfaces. The core may also be deformed in handling, cutting in segments, and in storage. Freezing of cores as practiced by some palynological laboratories can produce large ice crystals which can damage the core structure. In this context, it looks suspicious when all the magnetic excursions reported in the upper 30 m of Biwa Lake core (Yaskawa *et al.* *J. Geomag. Geoelectr.* **25**, 447; 1973) come from the top or bottom parts of defrozen core segments.

We would not like to imply of course that all local excursions of the Earth's magnetic field reported from cores are misinterpreted records of deformed layers. However, it is only fair to state that at present, no firm evidence exists to the contrary.

Only results reproduced in parallel sections should be taken as indicative of the behaviour of the Earth's magnetic field (Creer *et al.* *Earth planet. Sci. Lett.* **31**, 37; 1976). A layer with anomalous, but internally inconsistent magnetic record should be first checked in detail for anomalous sediment structure, sliced and X-rayed for example, before any attempt is made to interpret it as a record of past magnetic field. Duplicate or triplicate sampling of a core should be attempted whenever the diameter is sufficiently large to allow such a procedure. Performance of coring devices should be tested on recent sediments of similar consistency and size distribution as the studied layers.

Recovery of original magnetic record

The Koobi Fora study (Hillhouse *et al. op. cit.*) is an excellent example of problems surrounding the recovery of original remanent magnetisation. No universal palaeomagnetic technique is available at present that can guarantee a complete removal of post-depositional magnetic overprint and unambiguous identification of depositional polarity. This could become a serious problem in land-based deposits exposed to prolonged weathering and to the action of groundwater. Sediments deposited during intervals of reversed polarity may acquire fully normal remanent magnetisation during subsequent weathering episodes. Normal remanence of land-based weathered strata does not exclude their formation at the time of reversed polarity. Similar problems caused by remagnetisation were also indicated at the seafloor (Watkins *et al.* *Earth planet. Sci. Lett.* **24**, 113; 1974). Thus, the polarity corresponding to the time of

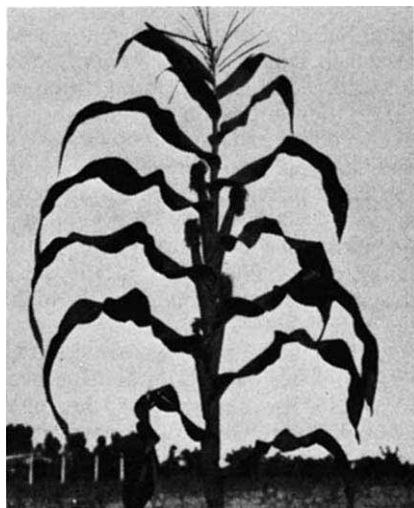
*F. P. Bonadonna, N. Bonhommet, E. von Braun, K. Creer, K. Kitazawa, G. Kukla, N. Opdyke, N. D. Watkins, I. Wendt, J. D. A. Zijdeveld.

A QUARTER of Yugoslavia's arable land is planted to maize, which vies with wheat as the single most important crop in Yugoslavia. A visit to the Maize Research Institute outside Belgrade proved an eye opener as to how a State Research Institute can be entirely self-supporting from the commercial sale of its products and expertise and at the same time support an active and directed basic research programme which complements its commercial and plant breeding sections. As a novice in this area I may have been over-impressed by the single-mindedness and enthusiasm of the workers at this Research Institute, applied to all stages of the production of a single crop—from research on seed production and gene banks to the extension services on the farm and the sale of seed and expertise (at home and abroad). The increasing interest in growing maize in the UK and Europe both for forage and grain has highlighted the versatility of this plant. It can be grown in a wide range of climates—all that is needed is to find a variety which suits the particular area and the purpose for which it is to be grown.

The Maize Research Institute at Zemun-Polje, about 15 km outside Belgrade, is situated on about 300 hectares of productive land, which is unfortunately now being encroached on by urban and industrial development. Modern well equipped laboratories have a pleasant working atmosphere in extensive fields around a central complex. The Institute employs about 250 people, a quarter of whom have university degrees, and is organised into three different divisions, a science division, an extension service, and an administrative and commercial affairs section. The extension service, which is involved in the controlled production of seed on large cooperative farms and which advises experimental farms, is the crux of the Institute's success. Nearly all the Institute's finance comes from royalties on the sale of its hybrid seeds, both in Yugoslavia and abroad. Nearly a half of its income is from abroad and it has close commercial and research links with more than

Maize in Yugoslavia

from D. O. Hall



20 different countries. Seeds are produced on about 20,000 hectares of good seed-producing land with a suitable climate.

The variability of climate in Yugoslavia from Mediterranean to Continental, its varied geography, and centuries of maize growing, have resulted in a unique maize gene bank. It is claimed that the Maize Institute has more than 100,000 different genetic combinations available for selection of new maize types. The storage of this gene bank is a major part of the Institute's service, since it provides a source of material not only for its own breeding programme but also for research work and hybrid seed production abroad. Yugoslavia seems to have been fortuitously placed in a favourable position for restocking the world's depleted gene banks, especially in North America where the widescale use of hybrid species has resulted in neglect of other varieties selected over the centuries. The Maize Research Institute is making the most of this advantage. Its sales of hybrid seed abroad and patented rights of seed production extend to 18 countries and these hybrids are now grown on over 1.5 million hectares abroad—not bad going for an Institute that only started to specialise in 1959.

Maize in Yugoslavia is mainly fed to cattle. More than 87% of the corn is converted into meat and Yugoslavia is a meat exporter. However fluctuations in the price of meat are resulting in a reassessment of the use of maize and new developments from the United States such as the production of glucose/fructose syrup and the utilisation of the whole maize plant for other types of product are being actively investigated. The potential for greater use of maize seems to be there. Over the past seven years the average yield of maize in Yugoslavia has doubled as a result of the use of the Maize Institute's hybrids which are now grown on two thirds of the total land area cropped to maize. They claim now that they have more than 75 new hybrids which will yield about 10 tonnes of grain per hectare as opposed to the average in Yugoslavia of 4 tonnes per hectare.

Research at the Institute is divided between four sections. Breeding and Genetics naturally employs the largest number of scientists, many of whom have been trained in North America. Research in the Physiology, Biochemistry and Technology Section takes several basic approaches which may have long term application to genetics and maize production. Low temperature growth in maize is being studied in the hope of extending the growing season by earlier planting and of understanding the physiology of low temperature adaptation. Developmental physiology and adaptation to the various climates found in Yugoslavia are also under investigation. Protein biosynthesis is being studied with the aim of increasing the lysine and tryptophan content of the plant. Biophysical assays which can be used for selection during breeding programmes are also being developed. The third section, Maize Protection, deals with fungal and bacterial diseases, insect attack and weed problems. The fourth, Agronomy, is involved in the techniques of maize cultivation. The integration of basic research and seed production and commercial exploitation seems in the case of this Institute to have been particularly successful.

deposition is to be considered an interpreted property of unequal accuracy, dependent on lithology, intensity of remanence, post-depositional history of the layer, the demagnetisation programme and the instruments used.

In these circumstances, the validity of magnetostratigraphic zonation of sedimentary rocks is only proved by reproduction of the results in sequences involving different lithologies,

depositional environments and sedimentation rates. Results of highest confidence are those obtained on stratigraphic successions of effusive volcanic rocks where the danger of remagnetisation is minimal. Magnetostratigraphers are urged to duplicate sampling in parallel sections and in parallel cores wherever possible. Further effort is also needed in studies of rock magnetism dedicated to recog-

nition of original remanence from overprint and to improvement of our understanding of processes through which the remanence of sediments is acquired and destroyed.

Correlation of magnetozones with palaeomagnetic chronology

Magnetostratigraphy is not a dating technique. It only provides globally synchronous correlation planes, whose

identification is possible by comparisons with other stratigraphic or radiometric data.

Local polarity zones represent valuable stratigraphic units independent of palaeomagnetic chronology which should be locally labelled and their boundaries clearly identified with respect to lithologic or biostratigraphic zonation. They could, but need not be correlated with the standard palaeomagnetic time scale. The time represented by the deposit is critical in the correlation. Is the deposit continuous? If so, what is the sedimentation rate? Does the thickness of the magnetostratigraphic unit fit the length of palaeomagnetic interval with which it is correlated? Does the reversal occur in a continuously deposited sequence? Or is it only an unrecognised hiatus? Many otherwise excellent magnetostratigraphic studies paid only marginal attention to the continuity and homogeneity of the sedimentary record. This resulted in serious miscorrelations, such as mistaking events for epochs (see Kukla & Nakagawa *op. cit.*).

The palaeomagnetic chronology scale is not a stratigraphic system since it was constructed from radiometrically dated samples of known polarity mostly without any knowledge of stratigraphic relations among the dated horizons. Under these circumstances, precision of K-Ar dating imposes serious limitations on the accuracy of reversal dating. Uncertainty about the true age of most reversals is substantial. Concentrated effort aimed at an improved accuracy of the palaeomagnetic scale by multiple radiometric dating of suitable layers in the stratigraphic vicinity of reversals is highly desirable.

As it stands now, the poor coverage of geological and stratigraphic aspects of studied sequences and the often insufficient removal of post-depositional overprint seem to be the principal weaknesses of magnetostratigraphy correlation. Most of the existing problems could be avoided or at least substantially reduced if repetitive sampling of parallel sections becomes a regular practice. □

Plants and animals interact

by Mary Lindley

An international symposium on Biochemical Aspects of Plant and Animal Coevolution was held by the Phytochemical Society of Europe in Reading on 4-6 April, 1977. The proceedings will be published by Academic Press.

CATALOGUERS of the chemical compounds found in plants are being stimulated to new thoughts by ecologists. They are looking for an explanation of how the compounds serve the plants and affect their interactions with animals. The members of the Phytochemical Society of Europe are closely involved and if evolutionary trends are slow to emerge, there seems to be no shortage of enthusiasm and ideas.

Volatile compounds are proving to be important in the pollination of orchids of the genus *Ophrys*, which resemble female insects. Male bees are induced to effect pollination while trying to copulate with the flowers, and some of the stimuli are odoriferous, as G. Bergström (University of Gothenburg) explained. He does not discount visual and tactile stimuli, but it seems that the morphology of the flower plays a small part in the process of attraction. It is not yet known whether the various volatile compounds identified in the flowers enable them to mimic the sexual attractions of the bees—more structures have to be determined,

particularly of cyclic terpenes.

The bees gain no nutritional benefit from *Ophrys*, but most of the interactions discussed involved the consumption of plants by animals, chiefly insects. Discussing the role of nitrogen in such interactions, S. McNeill (Imperial College Field Station, Silwood Park) reviewed evidence of a correlation between the abundance of insects on plants and the amount of nitrogen available as protein and amino acids. For example, the most numerous populations of insects on the grass *Holcus mollis* coincide with the peaks of nitrogen during spring and autumn. At the best of times the amount of nitrogen in plants is low compared with that of animals, and insects use various strategies to minimise the disadvantage. When it is lowest they may simply consume more material or move to a more nutritious plant or part of a plant. They might be able to modify the local physiology of the plant, for example by causing proteolysis, or they might turn to occasional predation on other animals as a source of nutrition.

The migratory and desert locusts (*Locusta migratoria* and *Schistocerca gregaria*) select the plants to give them the necessary nutrients through the effects of phagostimulants and deterrents. R. F. Chapman (Centre for Overseas Pest Research, London) explained that sugars, for example, stimulate locusts to feed, while such compounds as alkaloids deter them. It

seems that for a hungry locust the choice between different plants is determined by the presence of deterrents, while the size of the meal is more likely to be determined by the phagostimulants in the chosen host. Chapman suggested that two events in the evolution of plants particularly influenced the evolution of the locusts and their grasshopper relatives, the Acrididae. The first flourishing of the Acrididae seems to have coincided with the development of the flowering plants and the decline in the occurrence of condensed tannins, which are powerful deterrents. Later, with the coming of the grasses, the Acrididae could diversify again. Such examples, however, are unlikely to have been common in the evolution of insects, which Chapman stressed, has not been due on the whole to the influence of plant chemistry.

E. A. Bell (King's College, London) said that it has been a tenet of faith for him that plants make so many secondary compounds because they cannot run away from their enemies. The toxins in seeds include lectins, cyanogens, alkaloids and uncommon amino acids such as canavanine, but they are not all equally toxic to all animals. D. H. Janzen (University of Pennsylvania, Philadelphia) pointed out that the protection of seeds often needs to be more effective than that of leaves. Seeds contain a store of nutrients that makes them very attractive to predators unless unpleasant toxins are also present. To study the response of insects to various seed components, Janzen has been using synthetic seeds made from crushed seeds of the animal's host plant and incorporating into them toxins from other plant species.

On the basis of field tests, T. J. Mabry (University of Texas, Austin) believes that sesquiterpene lactones serve primarily as a bitter principle, discouraging animals from feeding. Deer and rabbits, for example, will not feed on plants containing such toxins, which occur most abundantly in the Compositae.

Participants did not all accept the view that secondary plant compounds always have a defensive role. D. A. Jones (University of Hull) feels that there is no absolute advantage in cyanogenesis, by which a cyanogen and the appropriate enzyme release hydrogen cyanide when the plant is crushed. His evidence was obtained with the polymorphic species *Lotus corniculatus* (bird's foot trefoil), which contains both cyanogenic and acyanogenic plants. Jones has found that on a cliff the greater tolerance of salt spray by acyanogenic plants is balanced by the higher potential rate of reproduction of cyanogenic plants. In close proximity,

in a hollow and on a mound, selective grazing by snails gives an advantage to cyanogenic plants. Clearly the situation is not as simple as it once seemed.

The traditional idea of a battle between insects and plants is also too simple according to H. F. van Emden (University of Reading). Drawing on his experience with aphids, he presented an alternative viewpoint. Insects such as aphids, which are restricted in their selection of host plants, might gain an advantage if the secondary compounds in the plants are primarily a defence against vertebrate predators. One benefit would be that both the insects and their food would be protected. □

Materials science and engineering

from Robert W. Cahn

"A GENERAL survey or reconsideration" is one of the meanings attached by the Shorter Oxford English Dictionary to the word *Review*. That usage dates from 1604, when modern science was in its birth throes. The natural philosophers of that time knew and corresponded with each other, and that curse and blessing of our day, the scientific journal, was as yet unknown and unnecessary. Today, the general survey or reconsideration is as essential to the working scientist as the Good Food Guide to the serious gourmet.

A review, of course, tells a scientist in a nutshell what the current state of knowledge is in a field, with extra emphasis on recent facts and insights. Who ever heard of a survey of ignorance? Yet that is what the editor-in-chief of the journal *Materials Science and Engineering*, together with his Advisory Board, set out to provide to mark the tenth anniversary of the journal's publication. The 264 pages of volume 25, dated September 1976, are made up of 35 short essays devoted to "what we do not know about" a range of subjects which fall within the scope of the journal. The tacit objective seems to be to guide the research worker who is contemplating a change of field, or of emphasis within a field, into fruitful problems. The editor invited a number of specialists all over the world to map the gaps and chasms in their knowledge. The result is unconventional, variable and useful.

One contributor complains: "Throughout my professional life I

tried to teach my students, when they were writing their first paper, not to elaborate too much what they didn't know. Of that, I told them, the future readers know enough themselves." However, the wise choice of expert contributors renders this review of ignorance acceptable and indeed useful. It is necessary to know a great deal before the gaps in that knowledge can be a source of wisdom. Nobody attempted to restrict himself to a survey of gaps: all, with greater or lesser terseness, first surveyed what is known. As another contributor expressed it—"I take it that we are asking: What principles governing . . . are generally agreed by the scientific community? What principles are controversial? What areas are unknown to the extent that no principles have been formulated?" This contributor achieved something very rare and indeed paradoxical in a scientific review: he included not a single reference to published work, but filled his own prescription in a discursive way, emphasizing the important points without documentation. The essay—L. M. Brown's on the Deformation of 2-phase Alloys—is a thorough going success. This approach can be followed successfully only with a widely familiar topic; one which is less familiar may need plenty of documentation, and yet effectively pinpoint the crucial areas of ignorance; D. A. Vermilyea's essay on Corrosion is a case in point.

Some reviews are highly theoretical and can only be followed by a reader who is already highly expert (for instance, H. E. Cook on Continuous Transformations; M. Wuttig and T. Suzuki on the Martensite Transformation); others are severely practical, addressed to specific industrial objectives (A. G. Chynoweth on Fiber Lightguides for Optical Communications; M. C. Flemings and others on Rheocasting; J. A. Manson on Modification of Concretes with Polymers). Most cover their subjects in breadth, some (N. Brown on The Effects of Gaseous Environments on Polymers; Flemings on Rheocasting) describe only their own work and the problems uncovered thereby, justifiably since they opened up new fields. One particularly illuminating survey, V. F. Zackay's on Thermomechanical Processing, is not so much concerned with gaps in scientific understanding as with the problems of finding a development strategy which will increase the industrial acceptability of the complex form of processing with which it deals. Altogether, the volume is an intriguing study in styles. In general, the broader the author's range of vision, the clearer his presentation. One might have expected that when subject to such severe constraints on the number

of words, the narrow specialist would have fared better, but indeed the general principle remains true that one sees the wood better from a helicopter than from the jungle trail.

The Editor does disclaim any attempt to cover the whole spectrum of materials science and engineering, but it is nevertheless true that coverage is lopsided. Nearly 80% of the reviews deal with metals and alloys, and within that category, there is heavy emphasis on the admittedly crucial topic of phase transformations.

It is nevertheless disappointing that only two essays deal with polymers (and one of these only does so tangentially), one with ceramics, and one with oxide glasses (again tangentially). There is no attempt to shirk topics in which understanding is unsatisfactory (E. Rabinowicz has plenty of ignorance to report on Wear, and does so without obfuscation), but no broad topic such as Materials in Biomedical Engineering is included.

In spite of the inevitable limitations and the uneven quality of the reviews, this volume deserves to be widely purchased by junior research workers particularly (the price is set low to enable them to do so). They should use it in conjunction with another compendium of useful short reviews, *Annual Reviews of Materials Science*, to gain a first-class overview of the direction in which the discipline is currently heading. □

A hundred years ago

A VERY extensive Etruscan necropolis has been discovered at Monteparo, near Ascoli-Piceno (Umbria). An enormous quantity of bronze, iron, and terra-cotta objects have been and are being found in the grounds, chiefly consisting of helmets, armillas, collars, buckles, nails, spurs, bows, rings, lances, spears, swords, and thousands of perforated bronze grains and beads, besides numerous objects of amber, glass, shells, and pottery, all of which are likely to be secured by the Italian Government for the Florentine Museums.

Two Prussian officers have arrived in Paris for the purpose of determining telegraphically the longitude of Berlin. Two French officers have been despatched to Berlin in order to carry out similar operations. The apparatus to be used in Paris have been located at Montsouris under the superintendence of M. Mouchez and the Bureau des Longitudes. The ultimate aim of the operation is to connect the French trigonometrical triangulation with the system of the Geodesical International Association which is covering almost the whole of Europe.
From *Nature* 15, 26 April, 560; 1877.

Book Review Supplement

IT HAS BEEN said that history is not made, it is written. It is written after the fact from fragmentary and confused memories, from interpretation of hearsay by people usually not involved, or by using indirect evidence to buttress a preconceived theory. This book is not such a perversion. It has the ring of authenticity, written principally on the spot and at the time by a man involved directly in the affairs reported. From my own experience in the White House with similar situations, it communicates a true sense of the dynamics, the conflicts, the frustrations, the personal friendships and animosities, yet the euphoria and satisfactions of advising a President on matters where he is inexperienced.

Dr Kistiakowsky has done a service by publishing his diary. As a diary, it focuses on events, not the process of science advising. As in a diary, specific subjects are fragmented temporally. For example, pieces of the story of the struggle for a nuclear test ban appear throughout the text. There has been no attempt to consolidate these except in brief comments at the beginning and end of the book. This is all to the good, in my opinion, for it lends reality to the account. Yet important issues themselves, such as machinations towards a civilian space programme, can be studied and documented. Scholars of events of that time will find the book indispensable.

For those interested in the process of science advising, the book will be a wellspring of information, even though the subject is hardly addressed directly. Indeed, to the perceptive reader, the process of advising (and the inevitable striving for influence) becomes starkly clear. The flow of events and the colliding opinions and egos document the most critical factor in science advising—knowing the President's deeply-held convictions and values (for example, as outlined above), and relying on them in advocating policy. Convictions and values of a President can sometimes be turned slightly by fact and persuasive argument, but not bent very far.

Also fascinating are the personal glimpses of President Eisenhower and his strong commitment to steps for peace. As a military man, he knew the fallibilities of the military, and their civilian bosses. He was well aware, too,

that budgetary conservatism must be leavened with values which look to the long-term future. He was, therefore, favourable to science and respected the judgement of the professional people involved. The opinions and *modus operandi* of a variety of prominent people both in and out of government are described and judged by Dr Kistiakowsky. Many of them may have rejoinders, if they are still alive and interested. A bit more about that later.

Advising a President

Edward E. David, Jr

A Scientist at the White House: The Private Diary of President Eisenhower's Special Assistant for Science and Technology. By G. C. Kistiakowsky. Pp. xvii+448. (Harvard University: Cambridge, Massachusetts and London, 1977.) £11.25.

The President's Science Advisory Committee (PSAC) emerge from the diary as a useful instrument, if somewhat obstreperous and self-possessed. The PSAC seemed to function best when it addressed topics at the forward edge of science of technology, but with modest immediate impact on budget, political, or social matters. The PSAC had its detractors on the political side in Dr Kistiakowsky's day as well as in mine. In later times, it became necessary to institute another mechanism—*ad hoc* panels reporting directly to the Science Adviser rather than through the PSAC.

The diary contains a number of significant truths, such as, "This alerted me again to the danger that a 'cold-blooded' scientist's opinion may be very poorly received by the public at large", referring to a PSAC panel's view on carcinogens in food. A larger, still vital truth emerges—that the government requires advice and counsel from knowledgeable private citizens at the top of their careers if it is to act wisely. It must create conditions in which both advisers and advisees are willing.

For me, the book brought back memories and stimulated reconsidera-

tion of my own experience in the White House. Perhaps most prominently, I am impressed that events have a startling continuity. Here, 17 years after the events described, many of the same issues are still before us. Only some of the players are different. For every topic that occupied Dr Kistiakowsky, I can recall a derivative topic that occupied me some 12 years later. He expended effort on the nuclear test ban, I on an extension of the ban and on SALT I. Principal players in his time were John McCone and Christian Herter; in my time, they were Henry Kissinger and David Packard. Dr Kistiakowsky was concerned about large space boosters, I about the space shuttle. He dealt with Keith Glennan and Herbert York, I with James Fletcher, George Low and Donald Rice (then OMB, now President, Rand Corporation). Dr Kistiakowsky worked on the carcinogenic content of cranberries and the rigid Delaney Amendment, I on the pesticide 2,4,5T and cyclamates (today, saccharin). His correspondent was Arthur Fleming, mine were Elliot Richardson and Charles Edwards. Dr Kistiakowsky tried to put forward an acceptable high energy physics programme particularly advocating the Stanford linear accelerator; I did likewise trying to keep accelerator facilities from budget-induced idleness, and I also advocated heavy-ion research and radio astronomy, including the X-ray spectrum. He dealt with Maurice Stans, I with George Shultz and Caspar Weinberger. Dr Kistiakowsky addressed the strength of Western *vis-à-vis* Soviet science, and that was a hot topic in my time too. Dr Kistiakowsky's time saw the demise of the U-2 and its surveillance of the Soviet Union. He also saw in the SAMOS programme—the beginnings of satellite surveillance as a replacement. This topic remained an important area for the science adviser until the end of my own term. The U-2 incident which 'spoiled' Eisenhower's tenure has led to a new era in which 'national technical means' is a technique accepted tacitly by both sides as insurance against military surprise. Thus, today's intelligence estimates can speak confidently about the size and character of Soviet military deployments and developments, even though the intent behind them is and will remain a subject of dispute.

But there were also new concerns in the early 1970s. Energy was a principal one, including the breeder reactor programme; so was health care, as distinct from biomedical research. (There was a concern in both areas about the performance and *modus operandi* of the NIH, a concern that persists at least in the political arena to this day.) Directed biomedical research, typified by the Cancer Crusade, became an unwise *cause célèbre* in the 1970s, beginning in the late 1960s under President Johnson. In my time, there was a social science focus on the adolescent maturation process. That concern was stimulated by the perceived decline in educational quality. There was increasing attention to social science research programmes and modelling as an aid to understand-

diary. Indeed, at least two of those people had entirely opposite perceptions of the same events from Dr Kistiakowsky's, based not only on their memories but on their own diaries. Clearly, there has been no attempt to deceive the reader in Dr Kistiakowsky's diary; rather, at work are the usual divergences of perceptions conditioned by participants' past experiences and personal egos, and the resulting preconceptions. Thus, the reader should realise that there are other sides to the stories as told in Dr Kistiakowsky's diary. A tell-tale sign is that Dr Kistiakowsky saw his judgments in the complex matters he described as almost infallibly correct.

Significantly, Richard Nixon as Vice-President appears several times in the book. The text of the diary may leave

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ing their workings (including the Limits-to-Growth convulsion). And above all, there was the concern about environmental pollution and the drive to control it. A central issue in my time was the pollution caused by automotive and coal-burning plants.

Some of these 'new' subjects had begun to surface in Dr Kistiakowsky's time. But like an older person whose eccentricities have become exaggerated, the Science Adviser's office, as it matured in the 1970s, became more and more concerned with these 'off-beat' topics, which ranged far beyond the earlier principal concern—the physical sciences. The roots of this trend are clear in Dr Kistiakowsky's diary. Some say this was the downfall of the office, but it wasn't. It was a necessary evolution to keep pace with presidential interests.

Beyond both latent continuity and its contrast with future events, the diary indicates clearly Dr Kistiakowsky's incisive judgments about issues and people, some of the latter not very complimentary. Undoubtedly, those people would have retorts to Dr Kistiakowsky's judgment of them. Furthermore, events are described in the diary as perceived by Dr Kistiakowsky at the time. It is reminiscent of the famous Japanese film, *Roshomon*. Its lesson was that each participant in a happening emerges with his own perceptions, very different from those of the others. Knowing that lesson, I took the liberty of talking with some of the other participants in a few of the events described in the

the impression that he was often on the 'right side' of the argument, and so somewhat enlightened. Yet, there is a second impression which emerges on careful reading—that Nixon knew how to judge an occasion and take an acceptable position. Dr Kistiakowsky, in his notes added last year to his diary, says he came into the White House with a negative attitude towards Nixon, found him self-centred, and "remained sceptical to the end of my stay in Washington."

This book is the most significant account of the first era of Science Advisers yet published. The post was established in 1957 and abolished in 1973, about six months after I left the White House. It was re-established by an Act of Congress, signed by President Ford in May 1976. The Carter Administration's appointee is Dr Frank Press, an eminent and capable Earth scientist. With his installation begins a new era of presidential science advice. I hope that during its span, even greater accomplishments will be added to those of the first era—a significant piece of which is recorded in Dr Kistiakowsky's book.

An accolade is due to Professor Charles Maier who encouraged the publication and added a highly useful introduction to set the stage for the otherwise cryptic entries which follow.

Edward E. David, Jr, was Science Advisor to President Nixon and Director of the Office of Science and Technology (Executive Office of the President) from 1970 to 1973. He is now a consultant, based in Chicago.

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Experimenta lucifera

Erwin Chargaff

The Professor, The Institute and DNA. By R. J. Dubos. Pp. 238. (Rockefeller University: New York, 1976.) \$14.50.

In the late 1930s, during my early days at Columbia University, I had frequent occasion to visit the Rockefeller Institute for Medical Research, as it was then called. The uninviting structures on Avenue A near the East River were not easy of approach. True to its character of a citadel of learning, the door of the Institute was fortified by gruffness; and after passing inspection by Cerberus or Cerbera, the visitor had to be accompanied to his destination by a special young man. In my case this destination usually was the laboratory of the organic chemist Max Bergmann, much admired by me. I had known Bergmann when he directed a Kaiser Wilhelm Institute in Dresden; here in New York he shared the uneasy fate of his generation of German emigrants. On seeing me, he would exclaim: "*Zuerst rauchen wir eine Friedenspfeife!*" producing a large glass jar in which a package of cigarettes was kept in a controlled habitat. Occasionally, he conducted me afterwards to another laboratory, that of P. A. Levene or D. D. van Slyke; and sometimes I would come upon the light-brown shadow of an elderly mouse-like figure tripping along the corridor walls. This, I was told, was Dr Avery, a name not unknown to me as that of the greatest expert on the pneumococcus, although at that time I could not have known how important his name would become to me a few years later.

These reminiscences were brought on by an uncommon book about an unusual man, Oswald T. Avery (1877–1955). It is, in my opinion, a very good book, and I enjoyed reading it. As the title indicates, this is not merely a scientific biography. The book operates on several intercommunicating levels, taking into account the man, the time, and the place; and painting, with extraordinary competence, the ever-changing human and scientific backdrops. This competence is not entirely surprising: René Dubos, apart from being a very good writer, was a member of Avery's department from 1927 to 1941, and the warmth of personal contact and observation is felt throughout the narrative.

Born to English parents in Halifax, Nova Scotia, Avery was taken to New York City at the age of ten, when his father, who was a Baptist clergyman, was invited to be pastor of the Mariners' Temple on the lower Eastside, even at that time a pretty horrible part of the city. But five years later, the father was dead, and the three sons were brought up by the mother who must have been an energetic lady. Oswald Avery, aside from becoming an accomplished cornetist, got a good education: first something called, soberly, the New York Male Grammar School, then Colgate Academy, Colgate University, and finally, between 1900 and 1904, the College of Physicians and Surgeons of Columbia University, which was then one of the best finishing schools for clinicians. The latter seems to have finished him in more than one sense: in went a lively, communicative young man, majoring in the humanities, excelling in oratorical contests, playing the cornet, leading the Colgate University band, and clearly not particularly attracted to the natural sciences; out came what I would term, perhaps with some exaggeration, a scientific recluse. This shock of confrontation is not a very rare event: I have noticed it often in several generations of medical students, when I taught at the same medical school; only few of those affected, I am sure, turned into other Averages; mostly they became psychiatrists or even psychoanalysts.

Scientists in general lead uneventful lives, with the exception of the few who, for instance, are guillotined during the French Revolution or killed by highway robbers in Southern France. What counts is their inner history to which their published papers afford only precarious access, for the history of ideas, and especially of scientific ideas, is a slippery discipline. But in the present instance, Dubos has succeeded in producing as multi-dimensional an image as is possible.

With the exception of a very short period, after graduation, in medical practice, Avery devoted his entire life to research in bacteriology and immunology, first in a now extinct private institution, the Hoagland Laboratory in Brooklyn, and since 1913 in the Rockefeller Institute, where he remained until he was 71. The last few years of his life were spent, let us hope serenely, in the South. Or as Dubos puts it: "In 1948, he decided that he had shot his bolt; as he no longer felt able to function effectively in the scientific arena, he retired to Nashville, Tennessee". Each of the verbs in the preceding sentence could lend itself to a philosophical analysis which I shall not attempt here, except to

wonder why society seems to reserve circus acrobatics and science for the very young, assuming that these occupations require muscular vigour rather than wisdom. Why had, for instance, Telemann not yet "shot his bolt," when at the age of 81 he wrote what is widely considered as his greatest work, the oratorio *Der Tag des Gerichts*? The multiplicity of possible answers shows that we have not yet found the correct one.

The major part of the book is devoted to a detailed and lucid discussion of the problems investigated by Avery during his 35 years at the Rockefeller Institute. This is done in five chapters the titles of which will indicate the areas under study: 'The Lure of Antiblastic Immunity and the Chemistry of the Host'; 'The Chemical Basis of Biological Specificity'; 'The Complexities of Virulence'; 'Bacterial Variability'; and 'Heredity and DNA.' It will be recognised that Avery was one of the early microbiologists who understood the dominating role that chemistry was to play in biology. This was, incidentally, quite in harmony with the *genius loci* of the Rockefeller Institute, which in this respect, as in many others, was a most remarkable place. Dubos does full justice also to this side of his story; and the Institute, with its members, seminars, conferences, and, especially, its memorable capitalistic-monastic lunch room, is one of the indispensable elements of his account.

Avery was comparatively late in starting, but he lasted: his most important work was published when he was past sixty-six. The pneumococcus was his microcosm; he showed that general principles of great import can be derived from little things if it is given to the researcher to join penetration to perseverance, and bold deduction to honest induction. As always, what counts is the balance, the mixture; but has anybody in science succeeded in mixing himself, in filling his own recipe?

Avery became interested in the pneumococcus because one of the principal projects studied at the Rockefeller Hospital was the development of a serum therapy for lobar pneumonia. I do not believe the practical results of his research ought to be stressed, but out of this work there emerged a new understanding of the chemical basis of antigenicity, and, even more surprisingly, the recognition that genes were made of DNA. These glories may be taken to demonstrate the stupidity of our era of target-directed research. Actually, science has never operated entirely without goals; but the goals were chosen by a few reasonable men, not by frightened politicians or bureaucrats, and were enforced with

Avery's Department, 1932. Seated (left to right): T. Francis, Jr, Avery, W. F. Goebel



tact and imagination. The directors of the Institute and the Hospital were wise enough to leave such a man as Avery in peace. They had trust in him; something that no 'peer group' with its silly priorities can afford or accomplish. The absence of frenzy is one of the main impressions I get from Dubos' description of Avery's laboratory.

The existence of many immunologically different pneumococcus types had been known for some time. In 1916, Avery's intimate friend, Alphonse Dochez—I knew him very well during his years as a Columbia professor—discovered that type-specific soluble material was released into the culture fluids by the organisms. These observations, extended and refined in the course of several years, finally led to Avery's collaboration with Michael Heidelberger, and later also with Walther Goebel, and to the identification of a host of type-specific bacterial polysaccharides as the basis of the immunological specificity of the various strains. It is not too much to say that this work had a profound influence on the growth of immunochemistry and on later concepts of "the chemical aspects of biological specificity." This was the title of the series of Jesup lectures that I gave at Columbia University four years after Avery's death. His name was mentioned more often than anybody else's, with the exception, of course, of my own.

Leaving aside a large number of interesting and important investigations by Avery and his collaborators—almost all within the confines of his 'pneumocosm'—I should like to move rapidly to what most of us will consider the most illuminating, the *luciferinum*, of his many *experimenta lucifera*. (Dubos quotes Francis Bacon's distinction, in his *Instauratio Magna*, between "experiments of light" and "experiments of fruit.") I refer, of

course, to the work on the biological activity of DNA. In view of the witches' brew now being stirred all over the world, with "recombinant DNA" as its main ingredient, I can only hope that the title of this essay will not have to be changed to *experimenta Luciferi*; although the Devil hardly needs experiments to make his point.

Less than sixteen years—1928 to 1944—were required for the first fundamental observation to lead to the definite proof that DNA was the instrument of genetic specificity. That it actually took much more time before this proof was accepted generally, was due to obtuseness, malevolence, and the desire to protect various vested interests. I remember the names of both the heroes and the villains in this story, but only a few of the first will be mentioned here. When the transformation of pneumococcal types *in vivo* was discovered in 1928 by F. Griffith, there were no loud objections, perhaps owing to the rapid confirmation of his findings in other laboratories or because most bacteriologists at that time were Lamarckians. But for some reason the observations were filed away and, had it not been for Avery, they might have slumbered a long time. It was in Avery's laboratory that Dawson and Sia achieved transformation *in vitro* and that Alloway described the isolation of a crude transforming factor. All this was accomplished before 1933; and Dubos takes great pains to explain why more than ten years elapsed before the next, and in every respect final, publication. Such explanations are really not necessary: before World War II, science was not yet an achievement sport; speed records formed no part of the accomplishments of a scientist as they do now. Griffith and Avery are both quoted as deprecating hurry; and Dubos tells us that Avery liked to recall "the words of an old black

patient who watched, with amused surprise, the young doctors rushing about the wards of The Johns Hopkins Hospital: 'What's your hurry, Doc? By rushing that way, you passes by much more than you catches up with!'"

When in 1944 the epochal paper by Avery together with Colin M. MacLeod and Maclyn McCarty appeared, it certainly was something worth waiting for. The stages leading up to this publication and the all-in-all shabby reception granted it by the experts are well documented in the book, although more could, and probably will, be said eventually. As to the effect that the identification of the transforming principle as a form of DNA had on me and on the direction of my subsequent work, I have described it before. Avery himself obviously realised the implications of his discovery much more profoundly than he was willing to put into print. The letter he wrote to his brother Roy on 26 May, 1943, is particularly instructive in this respect. His entire character as a scientist—relentless perseverance, courageous imagination, extreme caution—can be developed from this document.

Many readers will, I am sure, find one chapter especially moving—namely, chapter twelve, entitled "As I Remember Him." I have never seen this done before in the biography of a scientist. To the limited extent that a scientific investigator is also a human being, the carving of the private bust calls for an unusually tactful and sensitive observer; and this René Dubos must have been in the many years he spent with or near Avery. Nevertheless, Avery was an extremely private person, and there must have been a wall around him, not of his own building—a wall that constrained him as it restrained access to him. I am not sure that we can ever understand another man so as to resurrect him on paper. The reason why figures invented by great novelists strike us as so alive is that they are invented. At any rate, what I got out of reading about this shy, puritanical, disciplined, and cautious man was a renewed awareness of the poverty of greatness.

As I said at the beginning, this is an interesting book. It is also very well produced, with 22 illustrations, some quite fascinating, and with a good index. It should be read by all who consider themselves part of the 'biomedical community', and molecular biologists should read it twice. Even philosophers and historians of science, if they can spare a few moments from their contemplation of the dark side of the Reverend Moon, will find the book profitable. □

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Standing: E. E. Terrell, K. Goodner, Dubos, F. H. Babers

Conversations with Haldane

Eric Ashby

The Man with Two Memories. By J. B. S. Haldane. Pp. 220. (Merlin: London, 1976.) £2.80.

THE style is unmistakable; it has the taut logic of Handel's music. So is the puckish barbed humour: "As I was at a day school, I did not adopt homosexual practices . . .". And so are the sparks of exotic knowledge: "Of course I have a fair repertory of languages, including one or two unusual ones, such as Hittite, which I had to learn in order to study the earliest serious biological document, Kikkalish's instructions for training chariot horses." No-one but J. B. S. Haldane could write such stuff in such an enchanting style. Those who recollect his brilliant and sometimes exasperating conversation will find in this book a nostalgic recreation of what it was like to spend an evening in his company. Those for whom J. B. S. Haldane is a somewhat romantic myth—a man who could turn from mathematical genetics to polemics on sociology in the *Daily Worker*, and who used to leave his laboratory at the end of term to spend the vacation in the civil war against Franco—will get an impression of what it was like to be a contemporary of the man.

I remember him at a dinner of the Society for Experimental Biology. It was a light-hearted informal occasion and for some reason J.B.S. was asked to make a speech. In that grave, hesitating voice, as though about to begin a scientific lecture, he said that although he could not bring himself to believe in heaven, he now had some inkling of what heaven would be like if there were such a place. For he had greatly enjoyed this dinner, "thanks to the delectable company of the young lady on my right and the young lady on my left. Now in a three dimensional world one can have only two ladies sitting next to one. Heaven I believe, might be conceived to be, a place in n -dimensional space, where one could therefore expect at dinner to enjoy the company of $n-1$ young ladies."

This book is a mixture of J. B. S. Haldane's startling breadth of knowledge with his sparkling and often grotesque imagination. It is a fantasia with a purpose. The purpose is to expose by ridicule the prudery and bigotry and credulity of twentieth century western civilisation and to put on record glimpses of the sort of utopia he himself would have enjoyed. It is a work published years after his death. The blurb on the dust cover suggests that it may be regarded as a philosophical and

scientific testament. Anyone who opens it with this expectation will be disappointed. It has neither the cohesion or the conviction of a testament. It is a jumble of ideas, leaps of imagination, and exhibitions of prejudice which I don't think Haldane himself would have wished to publish. It has not got enough plot and movement to make it good science fiction, nor enough austerity of argument (and Haldane's scientific work was impeccably well argued) to make it good scholarship. Nor, I believe, did Haldane himself intend it to be in either of these categories. It is simply a feast comprising the after-dinner conversation of a man of extraordinary and fascinating personality.

The 'hero', James Robert Murchison, carries in his brain all the accumulated experience and response to experience of a British scientist. But there has also entered his brain and lodged there and become manifest to Murchison, all the accumulated experience of Ngok Thleg, a being from another planet. It is a case of memory transfer which can occur if two persons have similar genotypes. The planet from which Ngok's memory-and-experience bank comes has solved many

of the scientific and social problems which perplex planet Earth. The people there may multiply by clones; anti-social behaviour is promptly cured by injections of drugs; the unit of time is that taken for light in a vacuum to traverse 2.317×10^{17} wavelengths of the red hydrogen emission line; cities are built of an alloy of titanium and are enclosed from the atmosphere, kept at a 10% higher pressure with pumps and filters to remove bacteria (disease, therefore, was an anachronism). There is some interplanetary travel; some computerised information services; and a little highly aseptic permissiveness: all developments which have made some headway since Haldane's death.

The book is unfinished. It is not the sort of work which readers of *Nature* need feel they *ought* to read. But if anyone who is going by train from, say, London to Edinburgh would like to have J. B. S. Haldane's company for the trip, talking rather as he did to me when I made that journey with him some 40 years ago, this book will give him that experience. □

Eric Ashby (Lord Ashby of Brandon) recently retired from the Mastership of Clare College, Cambridge.

Father of ethology

Aubrey Manning

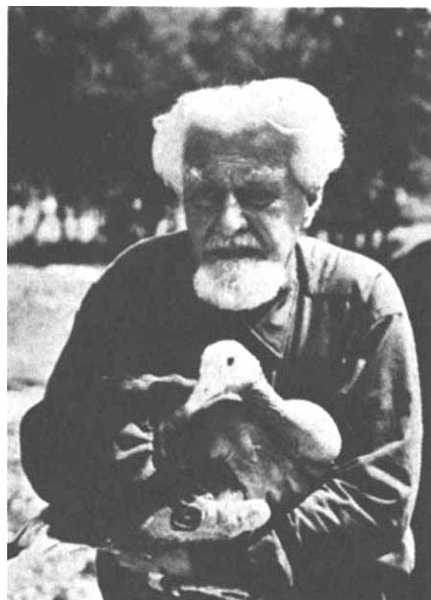
Konrad Lorenz. By A. Nisbett. Pp. xiv+240+21 photographs. (Dent: London, 1977.) £5.95.

BIOGRAPHIES of living people, particularly those around whom controversy has gathered, are risky ventures. It is usually better to let the dust settle so that the subjects are seen more clearly in relation to their own time and place. Konrad Lorenz is clearly such a subject but Alec Nisbett's book is successful in avoiding the main pitfalls: this is a thoughtful and substantial biography. Nisbett has a background in mathematical physics but made a career in the British Broadcasting Corporation, where he has won many awards for his skills in presenting scientific subjects to the general public. His interest in Lorenz arose from such work, and having obtained grant to work on this biography, he spent much time talking to Lorenz and some of his key associates. Nisbett obviously likes Lorenz and so does this reviewer. Consequently for both of us there is the danger of becoming indulgent and less than adequately critical when discussing Lorenz's ideas and influence. This does set out to be a critical biography but the soft pedal is certainly applied in some places.

Konrad was the younger son of Adolf Lorenz, a successful orthopaedic surgeon (he almost got the Nobel prize for medicine himself) who identified with the Austro-Hungarian aristocrats and built himself a large house in the baroque style at Altenberg near Vienna. (Konrad has returned to this same house in retirement.) Adolf wanted his son to follow him into medicine: he did qualify, but already the jackdaws, herons, ducks and geese had taken over his life. Lorenz lectured on animal behaviour in Vienna, but had no formal research facilities; the animals all lived at home. During this period he published all his most influential papers and began collaborating with Tinbergen. Academic recognition was crowned by Lorenz going to the prestigious chair of psychology at Königsberg (then in East Prussia). The war had already begun and soon enveloped the eastern front. Lorenz ended up practising simple medicine and writing on philosophy in a Russian prisoner-of-war camp.

He was not repatriated until 1948 when he finally got back to Altenberg to reconstruct his career. It was much harder to regain academic recognition in Germany. No university position was forthcoming although a host of new students were gathering around Lorenz. He worked more or less on an *ad hoc* basis until the Max Planck Institute enabled him to obtain facilities at Seewiesen in 1955. He retired back to Altenberg with a smaller research institute in 1973.

Lorenz tending a wounded goose



Nisbett provides an excellent account of Lorenz's life and skilfully manages to interweave comments on his scientific thinking. I had some contact with the developments in the mid-1950s, and he very successfully catches the atmosphere of the post-War emergence of ethology in Europe. There can be no doubt of Lorenz's key role in its birth. He is the father of ethology, however many of his children now reject his ideas.

The early papers in which Lorenz developed the concepts of the fixed action pattern, the releaser and the evolution of displays, of action-specific energy and of imprinting, are all the result of dedicated observation and description shot through with brilliant intuitive thinking. The approach to the animal in its natural environment and the concern with function and evolution came as a powerful tonic to behavioural science stultified by white rat experimental psychology. To read Lorenz's papers was enough, but to hear him lecture—the flood of words and ideas enlivened by his repeated assumption of diverse animal forms on the platform—was to experience instant conversion. Ethology took off, and although Lorenz himself is quick to point out his debts to Whitman, Craig, Huxley and Heinroth, it was he himself who founded the school.

Lorenz's own great period was over by 1960. He had little taste for the quantitative and analytical studies which had to come next—he boasts that no paper of his ever included a graph; nor can I recall any tables. The new impetus was provided by Tinbergen, who rapidly built up a group in Britain, and by groups in the USA.

Lorenz turned increasingly to the application of his original ethological concepts to human behaviour and his weaknesses began to overlie his strengths. He had always tended to

adopt rigid classifications in behaviour: instinct or learning, genes or environment. His disputes with the "English-speaking ethologists" as he referred to them, largely centred on their refusal to accept such dichotomies. Lorenz failed to appreciate that they were just as interested in the genetic basis of behaviour as he was, but often chose to approach the question through the study of development. It no longer remained helpful in such research to classify elements of behaviour according to any fixed scheme. When Lorenz began writing on the inherited basis of human behaviour he showed few signs of compromise. With the publication in English of *On Aggression* in 1966, the storm broke; Lorenz and those who supported his deterministic views on human behavioural development were ranged against the cultural and environmentalist schools.

As always in nature-nurture controversies, politics were to the fore. Lorenz's alleged Nazi sympathies during his Königsberg period were focussed on by the environmentalist left, especially when his Nobel Prize was announced. Nisbett provides a good account of the particular paper which gave such offence. He points out how certain mistranslations have been at the base of misunderstanding and misrepresentations. What Nisbett doesn't do adequately is to point out that whether or not Lorenz's views on the dire effects of domestication in man (whatever that means) were dressed up for Nazi consumption, they were in any case remarkably bad science.

Lorenz's reactions to the Nobel Prize furore saddened many of his friends. He admits himself that once on camera he becomes a buffoon. Instead of dismissing a youthful error at once, he tended to become defensive. He certainly gave a totally misleading impression at times. Nobody who knows Lorenz personally can doubt his warm humanity.

Nisbett's book will serve as an interim statement as it were. He gives a most useful list of references both to Lorenz's own work and to other writings which bear on his ideas. Perhaps the centre of gravity of this biography is inevitably towards the later period of Lorenz's career. Nisbett rightly feels that he must discuss Lorenz in relation to the controversies of recent years. When we come to look back at Lorenz's contribution in more tranquility, however, it is *King Solomon's Ring* that will stand out as his great book. Within its easy charm are all the important ideas which make up Lorenz's profound contribution to the study of behaviour. □

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Richard of Wallingford

Richard Lorch

Richard of Wallingford: An Edition of His Writings, with Introduction, English Translation and Commentary. Edited by John D. North. Three volumes: pp. 1,312; 22 plates; 128 text figures. (Oxford University: Oxford and London, 1976.) £75.

RICHARD OF WALLINGFORD (about 1292–1336) was among the ablest astronomers and instrument-designers of the Middle Ages. His *Quadripartitum*, for all its prolixity, bears comparison with Regiomontanus' *De Triangulis*, written a century and a half later; and his astronomical clock was one of the most advanced made in the fourteenth century. Again, the complex universal instrument 'Albion' ('All-by-one') was a remarkable achievement, and was fully appreciated by fifteenth-century astronomers such as John of Gmunden (about 1382–1442) and Regiomontanus (Johann Müller of Königsberg, 1436–76), who, among others, re-edited and commented on the *Tractatus Albionis*, in which Richard describes the instrument. Dr North has provided us with a reliable and annotated edition of all Richard's works. From this monumental edition, which must surely be ranked with Nallino's edition of Albategni, we may gain insight not only into Richard's achievements in astronomy, astrology, the making of instruments, and mathematics, but also into the progress of these sciences throughout the Middle Ages.

In studying any western astronomer of the high Middle Ages it is necessary to pay attention to borrowings from his predecessors, particularly the Muslim astronomers whose works were translated into Latin in the twelfth and thirteenth centuries. On the whole, Richard of Wallingford is explicit about his sources, but some problems remain. Thus, in the first part of the *Tractatus Albionis* he gives chapter and verse for each proposition, the usual sources being—besides Euclid and Ptolemy—Albategni's (died 929) *Opus Astronomicum* and an "abbreviated *Almagest*". As is pointed out in the commentary, the latter is better known as the *Parvum Almagestum*. This is probably a Latin compilation and has been attributed (by A. Birkenmajer, *Studia Copernicana* I, 143–6) to one Walter of Lille, although Campanus of Novara (died 1296) is also a possi-

bility. There are at least two variants of the *Parvum Almagestum*, quite apart from variations in the mainstream text, and one of these could well account for the remaining references to a "Commentator" on the *Almagest*. At all events, this Commentator is unlikely to be Averroës (1126–1198), whose commentary on the *Almagest* was apparently unknown in Latin (despite Birkenmajer's citation of the fourteenth-century Alphonse Dionysii de Lisbonne, who probably thought the *Parvum Almagestum* was by Averroës); and was so differently organised as to make the identification unlikely.

The sources of the *Quadripartitum* are not given with such readiness, but Dr North has discovered the most important of them: a text beginning "*Proportio est duarum quantitatum . . .*", which he for several reasons ascribes to Campanus. There are some valuable remarks in the commentary about the whole history of "cata" (Menelaus' theorem) and of ratios: it is to be hoped that these notes and the authors cited in them will encourage someone to make a critical edition of the main texts. The *De Sectore*, a re-working—apparently by Richard himself—of the *Quadripartitum*, is not printed in full. It is of some interest since it incorporates the new trigonometrical methods developed by the Muslim astronomers and introduced into the West by the translation of the astronomy of Geber (flourished during first half of twelfth century). Whether later English writers like Simon Bredon (about 1300–about 1372) copied Geber's methods direct or through the *De Sectore* is a matter for further research. What we do know is the approximate date of their introduction into England.



Richard of Wallingford (Cotton MS Nero D VII, f20)

Of the shorter works the treatise on the "rectangulus" is perhaps the most interesting. Characterised by Henri Michel as "*un véritable Torquetum schématisé*", the rectangulus was invented by Richard to "obviate the tedious and difficult work of making an armillary sphere". (Anyone who has made even a rough model of an armillary sphere will sympathise.) Dr North points out that such alternatives cannot be lightly dismissed as observing instruments, and further maintains the unfashionable view that they were also used for the transformation of coordinates. It is interesting that in both the *Rectangulus* and the *Albion* Richard follows the custom, found among Muslim writers, of listing the parts of the instrument, together with their technical names. Franco's treatise on the "torquetum" is a possible intermediary.

Some of the texts included in this edition—for instance, the canons to John Maudith's tables, with which volume 1 begins—are here attributed to Richard Wallingford for the first time; and the authorship of doubtful cases, such as the astrological tract *Exafrenon*, is fully discussed. The account of Richard's life, besides being interesting in itself, contains some useful remarks on biographical sources for Englishmen of this period. The texts themselves are mostly established from two or three good manuscripts (in some cases discovered by Dr North), the others being used in a subsidiary role. There are occasional omissions—no doubt following Bjørnbo's edition of Thabit's *De figura sectore*—of purely repetitive matter, but synopses are given to fill the gap. On the whole, the English translation, on facing pages, is literal; but, where appropriate, the material is clarified by the use of modern symbols and line-by-line layout of the argument. As one would expect from the author of *Kalenderes Enlumyned Ben They*, the Middle English translation of the *Exafrenon* is given in preference to a modern one.

The three volumes are conveniently arranged so that the text and translation, commentary, and diagrams, can all be seen at once. In volume 3 there are plates, indexes, glossaries, and no fewer than 39 appendices. Some of these appendices and some of the longer disquisitions in the commentary volume are complete in themselves, and form a collection of well-indexed articles on the periphery of the central theme. We can only hope that their author is not preparing to abandon the Middle Ages. □

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Newton as alchemist

A. Rupert Hall

The Foundations of Newton's Alchemy. By B. J. T. Dobbs Pp. xv+300. (Cambridge University; Cambridge, 1976.) £10.50.

EVER since David Brewster published his Victorian biography of Isaac Newton 120 years ago it has been known that Newton devoted much time to chemical experiments and alchemical authors; indeed, Brewster, while faithfully noting his hero's purchases of chemicals, apparatus and Ashmole's *Theatrum Chemicum*, lamented his waste of time on Ripley, Flamel, Starkey and the rest. Moreover, Newton's interest in these matters has long been evident from his correspondence. The dispersal of the great mass of Newton's private papers in 1936 simply made openly available a mass of material that had been formerly known but largely inaccessible, and which in any case had not been regarded as very important to those who were more interested in Newton as the founder of mathematical physics than as a man of his own age.

The mood has changed, so that during the past thirty years many have pursued Newton the whole man, rather than the author of *Principia*, *Opticks* and some mathematical treatises. 'Freudian' analysis has laid bare the mechanics of his personality, and he has been portrayed as theologian, historian, Mint Master, magus, and most recently as alchemist. How much light all this knowledge derived from deep reading and wide scholarship actually throws on Newton's role in the history of mechanics or optics is still an undecided question. The first attempt, by David Castillejo, to discern a unity in all Newton's work has remained unprinted (although copies are on deposit in certain libraries). In her new study of the earlier phase of Newton's interest in chemical topics Mrs Dobbs, after setting out the background that Newton inherited, investigates the kind of information about the nature of metals that Newton was looking for in his alchemical authors, and relates this to his own experimental endeavours. Finally, like Mr Castillejo (but more precisely), she explores the relations between alchemy, theory of matter and the concept of gravitation.

There is a difficulty in this aspect of Newton's work—for it was certainly a

serious enough activity to be judged work—which is encountered in studying Robert Boyle and others too. Newton actually published in his lifetime certain perfectly rational views about chemical properties and the nature of matter (which were not without influence on the development of chemistry as a science). We also possess factual and fairly straightforward accounts of experiments that he made on such and such days written in his own hand. We have again personal and authentic expressions of interest as in his letters to Aston (as early as 1669) and to Locke about Boyle's supposed incalcescence of gold with mercury (August, 1692). And in his London years, later still, Newton kept up an acquaintance with the alchemist William Yarworth. Finally, there is the known extent of his own alchemical library and of his voluminous extracts from alchemical authors, by no means excluding those of the esoteric sort. What is largely (but not, it seems, absolutely) absent is any comment, explanation, or reworking of this last material from Newton's own pen. His purposes, his intentions, his possible ambitions in collecting and studying it, have to be for the most part inferred.

As is evident from Newton's published chemistry, and from the experimental records first analysed by my wife and myself many years ago, Newton's mind in all this worked at a rational, factual, indeed quantitative, level. In this respect Newton's pyrotechnical work shows no difference of principle from his work in optics. If this were not true it is impossible to understand how *Query 31* or *De natura acidorum* could have been written. Much as we proposed nearly twenty years ago, Mrs Dobbs writes of Newton's translating alchemical processes into chemical terms which he could then subject to trial in the laboratory. If he plunged for enlightenment into the "most esoteric and mysterious productions of the alchemists", he sought their meaning by 'rational analysis', and experimented

methodically, almost ploddingly (pp168 and 175).

In Boyle's and Newton's day it was of course perfectly rational to suppose that metals might be compound bodies and capable of chemical modification. According to Mrs Dobbs, in a manuscript of his own composition headed *Clavis* (Key), Newton wrote out a detailed account of a process for preparing "a mercury dissolving all metals, particularly gold". (Its humourless author noted that "one philosophical sign will appear to you: in the very making of the mercury there is a great stink".) Such solution was preliminary to the complete mastery of metals. Like Boyle, the writer of the *Clavis* saw gold apparently fermenting and changing colour in this menstruum.

What is rather at stake is the question of Newton's commitment to the alchemists' ideas. Did their writings not merely contain hidden clues to actual procedures which could be verified or falsified in the fire, but a profound esoteric insight into the workings of nature? Here (as also in Mrs Dobbs' conjectural "school" of alchemy in Cambridge, whose leading activists were Isaac Barrow and Henry More, with Newton as their neophyte) it is less easy to accord with her views. It is true that Newton in *Opticks* and in the discussion of comets in the *Principia* hints at the transformation of a cosmic 'spirit' into water and so (as Boyle thought he had proved) into earth; such a progressive coalescence of the fundamental particles of matter (neither fermions nor bosons!) was not forbidden by contemporary theory, and had indeed been invoked by Descartes.

There are other ways too in which, as Mrs Dobbs rightly points out, Newton's faith in the "analogy of nature" led him into strange, or at least empirically unjustified speculations. But it seems needless to derive the concept of gravitational "attraction" (which Newton after all declared did not need to be an attraction and was certainly not inherent in matter) from alchemical origins, and extreme to affirm that "in a sense the whole of his career after 1675 may be seen as one long attempt to integrate alchemy and the mechanical philosophy" (p230). Are the mathematical papers in Dr Whiteside's eight volumes equally to be seen as of no significance in Newton's intellectual development?

Nevertheless, even the reader who may feel some reluctance to substitute *Caput Mortuum* for *Caput Draconis*, and some suspicion of any single key to the secret of Newton's greatness (many have been offered), must find his understanding of Newton broadened and stabilised by Mrs Dobbs' explorations of the more obscure recesses of his mind. □

Illustration shows the 'greene lyon' devouring vivifying celestial influences, symbolised by the Sun, and emitting a vivified mercury, the living or 'actuated' character of which is symbolised by blood.



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Scientific classic of history

J. D. North

Copernicus: On the Revolutions of the Heavenly Spheres. Translated by A. M. Duncan. Pp. 328. (David and Charles: Newton Abbot and London; Barnes and Noble/Harper and Row: New York, 1976.) £12.50.

JUST as in the Middle Ages the Bible and Liturgy were encased in Latin for the almost exclusive use of the priesthood, so in our own time most of the scientific classics of history remain either untranslated or—what is worse—badly translated. It should not be necessary to plead the case for a trans-

lation of the *De Revolutionibus* by Copernicus. The inadequacies of that prepared by Charles Glenn Wallis, and published in the series *Great Books of the Western World*, are easily exaggerated, but too numerous to make it an acceptable student edition. Dr Duncan's new translation is not only better, but it has the virtue of being separately available—that is, without an obligation to purchase the other 53 volumes of a collection.

Dr Duncan's style is modest, simple, and direct. His introduction could be readily understood by any sixth-former. The notes and introduction are not very long or profound, but neither are they likely to frighten away the inexperienced reader. The heliocentric idea is kept to the centre of the commentary, where it belongs, psychologically if not technically. The simplicity of the commentary is, however, occasionally misleading—as when the so-called theory of trepidation is considered, and as when modern disputes over Copernicus' Aristotelianism and the reality of the

spheres are deftly swept under the carpet. Copernicus's conservatism in the use of Ptolemaic methods and parameters is stressed, but Copernicus is represented as having made an advance on Ptolemy by the introduction of systematic observational tests of his model. The truth of the claim—simply because it is made so briefly in the introduction—is itself difficult to decide, but there are many who will find traces of special pleading in it, at least as far as it concerns Ptolemy.

The notes to the translation are slight and sparse, and the bibliography at the end even sparser. There are no indexes. The translation occupies almost nine-tenths of the book, and it is of course by this that the volume must primarily be judged. Speaking generally, the version is accurate and readable, and if it were possible to review the translation at pedantic length, most of my own objections would concern matters of taste rather than of substance. For example, out of kindness to the modern reader, *commutatio* is rendered as "parallax". On the other hand *obliquatio* is translated by the archaic "obliquation". I am not convinced that the flavour of the original is kept when *causa* is translated as "law" in such a context as Oslander's preface, as here: "... this subject [astronomy] is completely and simply ignorant of the laws which produce apparently irregular [planetary] motions". And I think that "correction" is an insufficiently specific rendering of *prosthasphaeresis*. But any careful reader of the text will not find much difficulty with technical terms.

The book is attractively produced at what must now be reckoned a very reasonable price, kept down by the expedient of printing the many figures in wide central margins. These are occasionally a trifle too small to be easily read, and are taken from the 1617 edition.

It must be noted that Dr Duncan's translation will soon be joined by another, produced by Edward Rosen for the Polish edition. The cost of the latter is likely to outstrip that of the David and Charles production, which is therefore more likely to reach the general reader, at whom the introduction and notes are aimed. Further comparison must be left until the Rosen translation is published. It is a moot point whether the market can take both versions, but English-speaking fans of Copernicus, working on the surgeon's principle that an extra opinion is always valuable, will no doubt insist that you cannot have too much of a good thing. □

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State of the Universe

Stuart Sharrock

The Key to the Universe: A Report on the New Physics. By N. Calder. Pp. 196. (British Broadcasting Corporation: London, 1977.) £5.95. *From Quarks to Quasars: An Outline of Modern Physics.* By S. Thomas. Pp. viii+294. (Athlone: London, 1977.) £3.95.

VERY few books deserve to be read. Some are memorable for the information they contain and others for the pleasure they give. It is a rare delight to find a book that fulfils both these criteria. On the whole Nigel Calder's latest offering, *The Key to the Universe*, is such a treat. At times the complexity of the subject is overwhelming and tedium sets in, but these occasions are few and forgivable. Most of the book manages to convey the sense of excitement and achievement so sadly missing in the majority of texts on physics. It must be admitted, however, that reading this book can be hard work—the very subject matter ensures this—but those who persist will be well rewarded. The great advantage compared with the television programme presented by Nigel Calder on the same topic, is that the concepts can be taken in at a leisurely pace and the reader can refer back to items which were incompletely understood at first sight.

The Key to the Universe is an attempt to explain in layman's terms the recent developments in elementary particle physics and astrophysics which promise to produce a theory of the workings of the universe. Beginning with electricity and rapidly plunging into the weak force and gauge theories, quarks, colour and charm, the story moves on to gravity and exploding black holes, the Big Bang and broken symmetries. These concepts are described in plain language and the text provides an excellent introduction to current beliefs concerning the origin of the Universe, how it works and how it is evolving. Only a Philistine could claim that this topic is not of interest; here it is presented in a manner which is accessible and enjoyable.

Like all good adventure stories there is a hero—the physicist. Not a single individual but a series of people doing experiments and propounding theories. The potentially obscure concepts are illuminated by this emphasis on the personalities behind the ideas. But the story presented is one of almost un-

qualified success, and consequently sounds at times a little contrived; it would be more realistic and gripping if the villains also made an appearance.

My major reservation is the fact that there are insufficient explicit statements about the relative standing of different concepts. Ideas are given more or less equal weight irrespective of their current status. The experimental basis for charm is rather stronger than that for the decay of the proton, for example, and the unification of electromagnetism and the weak force is considerably more advanced than unification schemes involving gravity. The unwary reader may not recognise this.

The initiated—whom are not synonymous with the intelligent—will no doubt find details which irritate. The muon, for instance, may well just be a heavy electron but it has had its own identity for decades; I find the label "heavy electron" bewildering and sad. But this is a minor level of complaint about an attempt to explain the workings of the Universe in 200 pages.

As a chronicle of recent achievements presented in an exciting and responsible fashion this book is unrivalled; the concerned reader will gain access and understanding to an important part of our culture, but not without hard work. This book demands to be read.

From Quarks to Quasars is an entirely different style of book. The suggestive title is misleading as only 10% of the text is concerned with astrophysical phenomena, and the message of the book is not the connection between theories of the microscopic and the macroscopic. No attempt is made to be up-to-the-minute—charm is still a dream of the future and black holes do not explode.

What is presented here is a competent introduction to the atomic nature of matter at an elementary and descriptive level; the basic concepts are introduced and applied to provide a useful summary of the various states of matter: gases, plasmas, liquids and solids. Properties of the nucleus and elementary particles are discussed almost as in a catalogue of 'useful facts', and the chapters on quarks and quasars are pure appendages.

This is a physics book containing a large amount of useful, well-ordered and basic facts presented at an elementary level in a readable but unexciting style. It is ideal background reading for budding physicists about to start a university course; the uncommitted will find it useful but uninspiring. □

Stuart Sharrock is Physical Sciences Editor of Nature.

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Remarkable progress in astronomy

Bernard Lovell

Astronomy Transformed: The Emergence of Radio Astronomy in Britain. By D. O. Edge and M. J. Mulkey. Pp. xvi+482. (Wiley-Interscience: New York and London, 1976.) \$31.75; £19.25.

HALF way through this book the authors state that they have completed their historical survey of the growth of radio astronomy. Their survey is primarily centred around the emergence of the new science in the UK covering the period from 1945 to about 1960—extended to 1963 to cover the discovery of quasars and, briefly, of pulsars in 1968. The authors state that they have chosen to base their description “around the development of research into discrete radio sources”, and within this restricted timescale and framework they have pursued their aim with great determination. Supported by extracts from taped interviews with many of the participants, and by a most thorough series of references to the literature, a detailed account has been given of fifteen years of remarkable progress in astronomy.

In 1946, J. S. Hey published the evidence that a localised source of radio emission existed in the constellation of Cygnus. He and his colleagues had used the receiver section of an Army defence radar, and no-one could have foreseen the extent of the technical developments which were to follow during the next two decades leading to the discovery of thousands of discrete sources of radio emission. Edge and Mulkey provide a fair and largely unbiased account of the succession of arguments and confrontations which by the end of their review period had led to a general consensus of opinion that the majority of these sources were extragalactic and that the measurements of their angular diameter and intensity distribution were of cosmological significance—even if the nature of the significance remained in dispute. After the pioneer work of Hey this story centres largely around the developments at Cambridge, Jodrell Bank and Sydney, and some of the participants will no doubt be surprised to be reminded how rapidly their opinions changed.

The meeting of the Royal Astronomical Society in October, 1951, was the occasion of a memorable exchange between Gold, Ryle and Hanbury Brown; and it is a pity that the full extract from this (like much other important material) has been relegated to appendices of notes and references which greatly increase the difficulty of a thorough study of the book. By 1951, although a few of the radio sources had been identified with extragalactic nebulae, Gold was almost alone in maintaining that the majority of the radio sources must be extragalactic. Even by 1957, when several thousand radio sources had been delineated, only eight had been identified optically; and three of these were associated with nebulosities in the Milky Way. By that stage the astronomical community was immersed in the contentious arguments about the differences between the Cambridge and Sydney radio source counts and whether they had relevance to the cosmological problem. Since Dr Edge was a graduate student in the Cambridge group for four of these remarkable years, he has been able to penetrate the details of a complex story, confused by the fact that, on the wavelengths then in use, the three strongest radio sources were near the galactic plane; but whereas two were supernova remnants in the Milky Way, the second strongest was a remote and peculiar extragalactic object.

The scope of the second half of the book is different. Here the authors investigate the relationships between the various groups of radio astronomers, the differing developments of the individual groups, the effect of radio astronomy on classical optical astronomy, and the extent to which the study of the emergence of radio astronomy contributes to the more general problems of the development of new areas of scientific inquiry. Parts of this will form compulsive reading for those who were in any way associated with these developments.

The bitter dispute over the radio source counts between the Cambridge and Sydney groups is summarised fairly. The remarkable secrecy maintained by Hewish and his colleagues over the pulsar discovery is described in relation to the problem of cooperation and competition, but the authors have balked the task of penetrating the Cambridge observational/theoretical conflict which has had such an influence on the present organisation of theoretical astronomy in the UK. In fact, their comment on this important matter is relegated to a footnote near the end of the book in which, referring to Hoyle, Gold and Bondi, they remark that the three “found themselves in Cambridge, but without any mean-

ingful relationship to the small observational radio astronomy group, with whom they found dialogue difficult”.

The most unsatisfactory feature of the book is the long chapter on *The Social Structures of the Groups* in which the authors “attempt to build up a picture of the evolution of the social structure and differentiation within the Cambridge and Jodrell Bank groups”. They do this in a most peculiar manner—from “publication profiles, and co-authorship and citation statistics”, and by quotation from anonymous members and ex-members of the two groups. The recollection of struggling research students of twenty to thirty years ago—however eminent they may be today—is scarcely a satisfactory means of making this investigation. In fact, I have difficulty in regarding this chapter seriously as a work of scientific or social investigation especially when after 57 pages the conclusion is reached that “the differences between the two groups can be adequately explained by postulating simply that Ryle and Lovell ‘are two different people’”. It is our view, however, that such a move is unnecessary”. We are different people and it is those differences embraced within a framework of mutual understanding and collaboration about the problems of development in a society with limited resources which have enabled both of our groups to survive and develop.

This chapter suffers in comparison with the following chapter on *Some Sociological Implications*. This is by far the most important and valuable section of the book in which the innovation of radio astronomy is placed in the historical context and compared with the emergence of other new scientific disciplines. Particular comparisons are made with Ben-David’s study of nineteenth-century innovations in medical science, of Mulkins’ study of the origin of molecular biology, and of the study by Dolby of the emergence of physical chemistry in the nineteenth century. This is a significant contribution to the scarce literature on a subject which should be of great concern to contemporary society, and is worthy of wider circulation than it is likely to obtain in this book. Clearly, Edge and Mulkey have condensed their information from an analysis of a great deal of published work and taped interviews; but further substantial sections could have been excised before publication, without detracting from a valuable historical narrative. □

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Optical astronomy

Bernard Pagel

Sterne—Kosmos—Weltmodelle. (Erlebte Astronomie.) By O. Heckmann. Pp. 353. (Piper: München and Zürich, 1976.) DM36.

THIS book is a collection of essays by the most distinguished living German optical astronomer and theoretical cosmologist of what is now the older generation. Scientific and autobiographical material is combined to make an unusually lively and lucid account in non-technical (German) language of the developments in which he has played a leading role: cosmology, positional astronomy, star clusters, development of the Hamburg Observatory (of which he became Director in 1941 after a four-year interregnum due to reservations as to his acceptability by the Nazi authorities), and the foundation of the European Southern Observatory (ESO, of which he was the first Director General from 1962 until his retirement in 1969). There are also shorter articles, including an obituary of the great Walter Baade, a Presidential address on the 50th anniversary of the International Astronomical Union and some experiences that read like short stories.

In the section on cosmology (described with deceptive modesty as the author's 'hobby'), the most interesting chapter provides an account of the history of the subject, his own involvement therein, and its relationship to German politics in the Nazi era. In the face of the official and noisy campaign against "Jewish physics", Heckmann held seminars on special relativity and on cosmology in which he took pains to expose the futility of such distinctions; and in 1942 (by which time that campaign had lost some steam) he published an important monograph on *Theories of Cosmology* which gave clear expositions of Newtonian, general relativistic and kinematic cosmology, but nevertheless got past the censor (perhaps because its arrangement so clearly brought out the connections between them), earning international acclaim. After the war, Heckmann continued to make important contributions to the foundations of cosmology until deflected by the departure of his collaborator, E. Schücking, and his own involvement with ESO. He gives a very clear and thoughtful description, enlivened with personal experiences, of developments up to the present, together with further chapters on historical and philosophical aspects.

Heckmann's chief personal contributions to optical astronomy were in the study of open star clusters (largely in collaboration with H. Haffner), notably the precision colour-magnitude diagram

of Praesepe. The astrophysical significance and the enormous work involved in reaching a degree of accuracy unprecedented in 1937, are very well described, although in this case the absence of any diagrams from the book is a weakness. At Hamburg, clusters continued to be a major preoccupation; another was positional astronomy—completion of the AGK2 and launching of the AGK3, which now provides proper motions and spectral types for 180,000 stars. Situated 20 km east of the city centre, Hamburg Observatory emerged from the War almost unscathed, although there was a narrow escape in the final battles around Lüneburg. After the War its staff was enlarged and its facilities enhanced with an 80-cm Schmidt telescope originally planned in 1937 in the hope of attracting Baade; it took immense political skill to get renewed funding in 1948. Significant studies of galactic structure were carried out with this instrument in the late 1950s, but its usefulness was already becoming restricted by city lights; and it has now been moved to the new Spanish-German observatory near Almería.

The ESO project was suggested in 1953 by Baade as a means of redressing the balance both between the Northern and Southern Hemisphere and between US and European optical astronomy; to save time and money, direct copies of Californian telescopes were to be used. Governmental support was rapidly secured in Holland, Belgium, Sweden and West Germany, and later in France and Denmark; and the Ford Foundation contributed a million dollars. Progress was slow, however, until the 1960s when the site of La Silla in the Andes was finally adopted after extensive site-testing in South Africa and Chile. It now boasts a fine collection of instruments including a 3.6-m Ritchey-Chrétien, together with offices and laboratories in La Serena, Santiago and Geneva—a far cry from the quick project based on existing designs envisaged by Baade, but nevertheless a great achievement which owes much to the energy and foresight of its first Director-General.

Heckmann's book gives a rounded picture of a man who has served the scientific community well through his thoughtful intelligence, integrity and very considerable courage, together with outstanding scientific and international vision, applied with great administrative and diplomatic ability. It is well worth reading, also, as an authoritative and often engagingly frank account of some of the most important developments in cosmology and optical astronomy in recent years. □

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Nineteenth century kinetic theory

N. Feather

The Kind of Motion We Call Heat: A History of the Kinetic Theory of Gases in the 19th Century. Book 1: Physics and the Atomists. Pp. xxxix+1-302. Book 2: Statistical Physics and Irreversible Processes. Pp. xxxix+303-769. By S. G. Brush. (North-Holland: New York and Oxford, 1976.) Dfl. 210; \$83.95 the set.

It is not until he reaches p168 of this weighty monograph that the author admits that its title is taken, in literal translation, from that of Clausius' paper of 1857 which, as he says, "defined the scope and viewpoint of most 19th-century work in the kinetic theory of gases". Nothing else in the monograph is similarly derivative. Since he was invited to deliver a seminar at Harvard in 1954, Dr Brush has immersed himself in his chosen subject, and has written copiously about it. The present publication is a re-presentation and up-dating of the contents of 17 articles (the author says 15) which appeared under his name (one with C. W. F. Everitt as collaborator) in various journals between 1957 and 1974. These have been organised in two sequences. In Book 1 are accounts of the relevant contributions of seven individuals, from John Herapath (1790-1868) to Ernst Mach (1838-1916); in Book 2 discussions of seven 'problems' which exercised the minds of nineteenth century scientists and philosophers—problems ranging from "the wave theory of heat" to "Brownian movement". These two sequences are prefaced by a 100-page "introductory survey", specially written to give coherence to the whole work.

This is an interesting arrangement; however, as the author concedes, it "obviously entails considerable redundancy". "That may not be a disadvantage", he adds, optimistically, "... the reader can jump into the ... chapters in almost any order ... without losing too much of the overall picture". Unhappily, such licence is not vouchsafed a reviewer—although when he meets an outline of the derivation of Boltzmann's H theorem for the third time he is tempted to skip.

The chapters are divided into sections, and footnotes are collected together at the end of each section. There

are some 108 sections so annotated, and with the number of sectional footnotes ranging from 1 to 59, it will be clear that the publishers' claim that "the author's historical researches have gone far beyond any other books on the subject" has at least statistical justification. Moreover, the last 49 pages of the monograph contain a separate bibliography, with brief indication of content, of 539 publications, listed by authors arranged alphabetically. These publications, Dr Brush considers, constitute the essence of the matter, so far as the nineteenth century is concerned. He has cited "thousands" of others in the footnotes—but he admits that they are "minor works".

What, then, has this monumental effort achieved? A balanced account of the history of the development of the subject within the century surveyed—that cannot be gainsaid—and a re-distribution of emphasis and credit. Credit to Herapath and Waterston for being in advance of their time (largely because the notion of heat as a wave process, which became popular in the early years of the nineteenth century, went far to diminishing the impact of the experimental researches of Rumford and Davy). Similar credit to Leslie as the first to distinguish the three modes of heat transfer. Credit to Loschmidt and E. P. Culverwell for pertinent criticisms which led Boltzmann to a deeper study of his statistical theory. And, in respect of the last years of the century, a re-appraisal of the discontents which beset kinetic theorists generally—the problem of gaseous specific heats seen as a minor problem, in comparison with more fundamental doubts of a quasi-philosophical character concerning randomness and irreversibility.

No-one will read Dr Brush's account without having his horizons widened and his detailed knowledge of the history of the subject immeasurably increased. It must be said, however, that he is likely to be much irritated in the

process. Leaving aside the 'redundancies' in text and footnotes (it would seem almost as if the field is wide enough to sustain an ergodic theorem in respect of the continuing theme of this book), he will be irritated by the numerous textual errors and misprints.

As a matter of interest, I checked the examples noticed during a single reading of chapters 11 and 12 against the relevant articles in *American Journal of Physics* where the material was originally published. I found three cases in which a typographical or textual error was repeated from the original version, and fourteen examples of errors newly appearing—five in new matter, and nine in places where there had been no error before. One example of the latter class is particularly puzzling. On p413, the factor $(1+(b/v))$ in equation (13)—which was correct—has been replaced by $(1-(b/v))^{-1}$, although the whole point of the argument in the following paragraph turns on the retention of the correct form in the equation.

There is an interestingly gratuitous error in the introductory survey (p43). Traversing a field of physics less familiar to him than gas theory, the author is presenting the old story of Fresnel and Poisson and diffraction by a circular disc. Poisson, he says, concluded that, if Fresnel were correct, "there should be ... a tiny bright spot at a certain distance behind the disk". When the test was made, "the bright spot appeared just where the mathematics predicted".

In a work of the magnitude before us, occasional errors are inevitable, but, sadly, there are more than they should be; and some will almost certainly mislead. American usage, too, is sometimes subtly different from our own. One example will suffice. Cayley is described as a 'colleague' of Tait (p358). Cayley, however, had left Cambridge before Tait went up as an undergraduate; by the time he returned, Tait was already in Edinburgh. They were never workers in the same institution. This usage, however, seems not to be accidental. In his preface, Dr Brush has a list of 22 "colleagues", the world over, whom he thanks for "valuable suggestions and information". At least he does not call his helpers "co-efficients", as Kelvin did (preface, *Baltimore Lectures*). Incidentally, Kelvin's nephew, J. T. Bottomley, worked, not "in England" (p520), but in his uncle's laboratory in Glasgow—and Oliver Lodge published (in 1894), neither from London nor from Birmingham (p745), but as professor in Liverpool. □

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Perspectivist tradition leads men of vision

J. D. Mollon

Theories of Vision from Al-Kindi to Kepler. By D. C. Lindberg. Pp. xii+324. (University of Chicago: Chicago and London, 1976.) £13.60.

In laboratories and coffee-rooms it is countless times debated whether a knowledge of the literature allows the scientist to stand on the shoulders of his predecessors or merely bestows on him a *damnosa hereditas*, fatally constraining his thought. The literature man scorns the rediscoveries and the omitted controls of his unread colleague: the latter argues that it is often faster to do the experiment than to find the answer in the literature and points to the low publication rate of the literature man.

Two of the greatest contributors to our understanding of visual perception, Thomas Young and Hermann Helmholtz, were literature men of enormous erudition whose systematisations too easily conceal from us the achievements of the eighteenth century. In his book, D. C. Lindberg argues that a third outstanding innovator in the theory of vision, Kepler, was not a revolutionary working in a vacuum but rather represents the culmination of the perspectivist tradition that extends back to the Islamic writers of the ninth and tenth centuries. He shows that Al-Kindi first made explicit the principle that luminous rays issue in every direction from every point on the surface of a luminous body and that Alhazen set the problem, that of establishing a one-to-one correspondence between points in the visual field and points on some plane in the eye. The pre-Keplerian solution was to assign an especial potency to those rays that were perpendicular to the surface of the cornea, that is, to those making up the visual cone, with its base on the object of vision and its apex at the centre of curvature of the cornea. (Lindberg does not notice that this theory is lent some truth by the Stiles-Crawford effect, the directional selectivity of the photoreceptors that favours rays normal to the retina). Kepler, equipped with the improved anatomy of Felix

Platter and with his own understanding of the focusing properties of transparent spheres, was able to offer the correct solution, that the crystalline humour is not itself the seat of vision but in combination with the cornea serves to form an inverted image on the retina. But, Lindberg argues, the perspectivist tradition gave Kepler his problem, his facts, his methodological rules and his criteria of success. In this conclusion Lindberg explicitly opposes the standard view that Kepler transformed visual theory by mechanising it: "Kepler presented a new solution (but not a new kind of solution) to a mediaeval problem, defined some six hundred years earlier by Alhazen".

Lindberg himself is clearly a literature man by temperament. His impressive grasp of the primary Latin texts is complemented by a very wide knowledge of the academic literature: his book has over 1,000 footnotes to secondary sources. So it is understandable that he is unimpressed by Leonardo's contribution to visual theory. Leonardo was avowedly not a literature man and himself wrote: "If indeed I have no power to quote from authors as they have, it is a far bigger and more worthy thing to read by the light of experience, which is the instructress of their masters. They strut about puffed up and pompous, decked out and adorned not with their own labours but by those of others, and they will not allow me my own". Lindberg believes that Leonardo had read Pecham and Witelo but that his knowledge of the optical tradition was imperfect. Usually Leonardo did not work with Al-Kindi's principle of punctiform analysis but rather thought of the species, or image, of an object as radiating holistically. His understanding of the formation of images by reflection and refraction was limited. It is particularly curious that this skilful anatomist was so weak in ocular anatomy: he confounded the vitreous and the crystalline and supposed that the eye consisted of two concentric spheres. By reading any of the mediaeval perspectivists he would have been furnished with a more sophisticated account. Lindberg is mistaken, however, in admonishing Leonardo for claiming that the apparent size of objects increases with the size of the pupil: there is a clear basis for this observation in the phenomenon of irradiation. Leonardo's *explanation* is another matter, but it is noteworthy that his claim about the effect of pupillary size is contained in the same manuscript (F of the Library of the Institut de France) as his observation that a uniform iron rod heated only in part of its length will appear considerably larger

in its heated part. The latter phenomenon is primarily a consequence of irradiation.

The theory of optics and vision was central to mediaeval philosophy and provided a link between physics, medicine and theology. Optics was, for example, fundamental in Bacon's planned synthesis of all human knowledge. Lindberg's book should therefore be of interest to mediaeval historians in general. He draws attention, for example, to the neglect of the history of the translations from Greek and Arabic to Latin in the twelfth century: almost nothing is known of the purposes and motivation of the translators, of the selection of works or of the mechanics of translation; and towards the further understanding of this crucial movement, Lindberg provides a detailed Appendix on the translation of optical works.

The book is illustrated with many diagrams of the eye drawn from ancient and mediaeval authors. Man's idealised representations of his own visual organ serve nicely to illustrate how his perception is influenced by his conceptual framework. Perhaps we can never accurately perceive a biological mechanism until we have constructed the artefact that will serve as a model: we must have lenses before we can perceive the eye as an image-forming device and computers before we can perceive the brain as an information-processing machine. What is regrettable is that Lindberg nowhere includes an accurate modern diagram of the eye, an inclusion that would have been invaluable to the general reader trying to follow mediaeval discussions of whether there are four tunics or seven, three humours or two.

To reduce his material to manageable proportions, Lindberg omits discussion of theories of the physiology and psychology of vision. To some extent this exclusion is artificial, for it is clear that many of Kepler's predecessors (including Leonardo) were held back from the correct solution by explicit reluctance to entertain an inversion of the image; and Kepler himself acknowledged that he "dutifully tortured" himself "in order to show that the cones intersecting when they pass through the aperture of the uvea intersect again behind the crystalline, in the middle of the vitreous humour, so that another inversion is produced." But he protested that geometry allowed no escape from an inverted image and thereby bequeathed to his own successors a new and peculiarly vexatious problem. □

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Mental imagery

John Krebs

The Question of Animal Awareness. By D. R. Griffin, Pp. viii+135. (Rockefeller University: New York, 1976.) \$8.95.

GRIFFIN'S thesis is that animals experience mental images and therefore a primitive form of awareness. One could argue about the meaning of these words and their relationship to the more strictly human experiences of subjective emotions and self-awareness, both of which are specifically excluded from Griffin's definitions. But since it is easy to see roughly what Griffin is getting at, I think such a philosophical argument is superfluous. Much of the book is filled with the 'evidence' for animal awareness, and although the style is clear and concise, I feel that Griffin makes an unnecessarily elaborate case: most behavioural biologists are quite ready to accept the idea that animals have mental images of events or objects in their environment.

Th main points of Griffin's thesis are as follows. First, he establishes, with a few examples—such as bats

catching their prey by sonar and birds orientating by star compasses—that animals are rather more complex than might have been supposed 50 years ago. Especially remarkable are the linguistic abilities of chimpanzees and honey bees. Chimps, such as Washoe, can construct sentences in American Sign Language, showing that they understand a few rules of human language, and bees use an arbitrary communication system for encoding information about remote feeding places.

Griffin sees the complexity of animal language as important, because so much of man's mental experience is dependent on language. (As an aside, the chimp and bee examples are always quoted as evidence for the complexity of animal language: chimps one can understand, but bees seem to be an anomaly. Either they are an evolutionary freak, or we are waiting for more scientists of von Frisch's genius.) In fact, the complexity of animal language does not seem to be a crucial part of Griffin's thesis, since some of the clearer examples of mental imagery in animals—for example, 'searching images'—do not involve complex language.

Another line of evidence used by Griffin is neurophysiological. He revives the argument that the nervous system of man is only quantitatively different from that of animals, so products of the nervous system such as awareness should also differ only in quantity. This assumes, of course, that quantitative differences are not translated by thresholds into qualitative effects.

Finally, Griffin argues on evolution-

ary grounds that mental awareness has survival value in allowing an animal to anticipate and cope with complex problems, so it should be favoured in all animals by natural selection. Interestingly, Griffin does not use dreaming in animals as evidence for mental imagery, although all dog-watchers will agree that dogs dream about chasing the cat next door.

Whether or not they accept the individual steps in his argument (most of which are not new), most behavioural biologists will concur with Griffin's overall thesis. Equally, I feel that most will be disappointed with Griffin's answer to the "so what?" question. The test of the value of any scientific hypothesis is whether or not it generates new experiments, and it seems to me that the awareness hypothesis fails this test.

Griffin argues, by analogy with man, that the only way to find out about an animal's mental experiences, it to ask it directly, or indirectly, what is it thinking about. With Washoe this might be done through American Sign Language, with bees and birds through life-like dummies or models. If one leaves out the special case of Washoe and his clan, what it boils down to is a plea for more work on animal communication. This is an uncontroversial proposition, but if I try to think up experiments or observations to elucidate the meaning of, for example, the incredibly complex song of a skylark, is the hypothesis that larks have mental experiences going to help me? □

John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

a revolution in the theory of gravitation
**A NEW RELATION BETWEEN
 THE VELOCITY OF LIGHT
 and PLANETARY MOTIONS**
 discovered by
MORRIS REDMAN SPIVACK
 PART II of

$$G = \frac{Mm}{r^2} \frac{c}{\dots}$$
 (A revision of
 NEWTON'S LAW)
 (5th EDITION—JAN., 1977)

Abstract:

1. GRAVITATION transfers energy from one body to another with the velocity of light. The relative intensity of the field controlling the orbit may therefore be expressed as a proportion of the velocity of light depending on the radius between the bodies.

2. In the case of the non-periodic advance (excess motion) of the perihelia of any planets — if the excess motion be known (as it is in the case of MERCURY), then dividing the excess motion by the observed mean orbital motion will give a PROPORTION of the RECIPROCAL of the velocity of light (as pure number) which can be obtained independently when the non-periodic advance is not known (for example in the case of MARS).

3. The MEAN MOTION when multiplied by the proportion of the reciprocal of the velocity of light ("c") thus independently obtained, will ALWAYS give us the EXCESS MOTION — and vice versa as at (2) above.

4. The METHOD of obtaining the correct proportion of "c" independently and directly from relativistic principles is demonstrated herein.

5. A new relation between the velocity of light and planetary motions is thus established, which may be useful in solving other problems of illimitable range and most certainly lead to the discovery of other important relationships and equivalencies in relativity.

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Brain anatomy

J. Z. Young

The Vertebrate Brain. By R. Pearson and L. Pearson. Pp. xi+744. (Academic: London and New York, 1976.) £23.

THE great expansion of interest in neuroscience in recent years has not been accompanied by an equivalent interest in comparative neurology. Microscopists and experimentors have indeed used neurones from very diverse animals, but few have tried to investigate their specific adaptive features in relation to the environment. Still fewer have tried to follow the course of evolution of the brain throughout the vertebrate phylum. The only comprehensive work on the subject is that of Kappers, Huber and Crosby written in 1936. The leading living expert in the field in Hans Kühlenbeck, whose major work is still appearing. One looks therefore with excitement at a new book on *The Vertebrate Brain*.

The authors' aim is to provide "a survey of the anatomical, biochemical and physiological aspects of the vertebrate brain". They believe that the development of the neurosciences "will probably prove as profound in their effects on man and his philosophies as did the concepts of nuclear physics during the 1930s or the 'molecular biologists' of the 1950s".

This may turn out to be so, but unfortunately the data that they have collected certainly do not provide a sufficient basis for such a revolution. The function of the nervous system is to process "information", but this word does not occur in the index, nor does "code" or "representation", or indeed "action potential", "electroencephalogram", or most of the terms of neurophysiology. We shall only be able to compare different brains when we have developed adequate ways of analysing how complex masses of nervous tissue process information.

What the present authors have done is to make a survey of some aspects

of the literature of the classical comparative anatomy of the cell masses and tracts in different vertebrate brains. It is a formidable problem, indeed, to know how to present this knowledge. The authors have decided to take each part separately. So we go through the details of the medulla from Agnatha to Mammals and then start on the cerebellum, and so on. It is not the authors' fault that such a division makes for difficulties in following particular functional systems. In some parts it works well—for instance, the mid-brain roof—but it

is disastrous for the reticular system, which is scattered all through the book, and it does not work well for the fore-brain.

No-one has discovered the principles with which knowledge about the structure and functioning of various nervous systems can be compared. The authors' brave attempt provides some of the facts and the stimulus to attack the problem. □

J. Z. Young is Emeritus Professor of Anatomy at University College, London, UK.

Perceptual exploration

Stuart Sutherland

Cognition and Reality. By U. Neisser. Pp. xiii+230. (Freeman: San Francisco and Reading, 1976.) £3.70.

In 1967, Neisser published a book called *Cognitive Psychology*: it was a vigorous and clear account of that subject which marked its coming of age. The book was optimistic and gave the impression that we would eventually come to understand the workings of the mind in terms of the information processing operations it carries out. In *Cognition and Reality*, Neisser seems to have become somewhat disillusioned with the progress of the cause he espoused ten years ago. Workers in the field, he claims, are trapped in specific experimental paradigms; they fail to tap the true potential of the human mind since they use unpractised subjects and artificial tasks; their work has little bearing on real life situations and does not tell us what mind or consciousness really are.

Neisser's own solution to the problem of cognitive psychology takes the form of a shot-gun marriage between J. J. Gibson and F. C. Bartlett. To understand the mind, we must first understand the world in which people live: in particular we must understand the relationship between the information available to our senses and the external world specified by that information. The information we "pick up" depends on our internal schemata: they guide perceptual exploration and are in turn modified by it. Perception is not an instantaneous happening but an ongoing interaction between our schemata and the world. "Images are not pictures in the head, but plans for obtaining information from potential environments".

The book makes some cogent points even if they are often incidental to the main theme. For example, Neisser points out that except in a very con-

strained environment where people have few choices, we shall never be able to exert behavioural control over other people: to do so, we would need to have a complete understanding not only of them, but also of the world in which they live. We cannot predict, let alone control, the moves a master chess player will make unless we have at least as much knowledge of chess as he does. Elsewhere, Neisser exposes some of the crudities of earlier theories of cognition. He has himself shown, amongst others, that with sufficient practice it is sometimes possible to carry out two complex tasks at once: the brain is not in any obvious sense a limited capacity channel.

Cognition and Reality is not an easy book to understand or to evaluate. First, it is unclear how far Neisser is putting forward a new thesis rather than merely using a different terminology. Nor is it obvious whose views he is attacking. There is a good deal of verbal fencing: for example, he registers his objection to the idea that the mind processes information by writing: "The information itself is not changed, since it was in the light already. The schema picks it up, is altered by it, uses it". Second, his exposition of the operation of schemata remains at a very general level and he is curiously reluctant to give examples of how his ideas can explain any phenomena in detail. When he does attempt explanation, as, for example, in his discussion of the perception of emotional expression in others, the explanations are at such a general level that it is hard to see whether any new light has been thrown on the problem.

At times one may even suspect that the constant flourishing of the magic word "schemata" serves more to distract attention from the phenomena to be explained than to explain them. Although the status of Neisser's central thesis remains unclear, the book deserves to be widely read if only for the interesting and acute observations that are scattered through its pages. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex, UK.

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Technology and food production

C. R. W. Spedding

Food Production and Consumption: The Efficiency of Human Food Chains and Nutrient Cycles. Edited by A. N. Duckham, J. G. W. Jones and E. H. Roberts. Pp. xx+541. (North-Holland: Amsterdam and Oxford, 1976.) Dfl.120; \$48.95.

THIS is a bold and ambitious book that deserves to be treated as a serious attempt to encompass in one volume the entire food production system of the world, from photosynthesis to marketing and consumption. Rigorous criticism is therefore a better use of limited reviewing space than mere description of contents. The latter can be summarised as being organised into 28 chapters (by 31 authors) in five parts (introduction; ecological aspects; biological components; social and economic aspects; a strategic synthesis). The book is well produced and edited, and relatively free from minor errors.

The quality of the individual contributions is inevitably uneven but as a compilation of basic facts the book is extremely valuable, although more could usefully have been included on, for example, the inefficiencies of animal populations and nitrogen fixation in plants. The enterprise, however, was intended as much more than a compilation; what has to be assessed, therefore, is the success of the synthesis achieved.

Energy is used as "the major currency" and, although the arguments are strong, it is perhaps rash to base a total synthesis too heavily on this alone. It is argued that the value of land depends largely on its ability to derive an income from solar energy. The world is, however, full of exceptions to this: the very ability referred to is dependent on other inputs with costs that have to be justified by probable profit and this is often not related at all to current energetic efficiency.

Furthermore, there are dangers in regarding food as merely a biological necessity. Space, water and oxygen are also biological necessities, but who would accept an argument based on providing the minimum biological requirements for these necessities? If man wants more than the minimum out of life, 'better' food would seem at least as reasonable an optional extra as cars, bingo or television.

Indeed, the book recognises that there is little hope of diverting cereals from livestock even though this could result in more food being available. The question of "available to whom?" remains a worrying one. The confidence generated by the conclusion that there are "no insuperable biological problems in feeding the world up to 2000 AD" evaporates somewhat when it is realised that both hunger and over-consumption now exist independently of technical knowledge, even when applied.

The conclusion that food systems are extremely inefficient is well documented but there is, in my view, too little emphasis on the problems of tackling such inefficiency. Identifying a problem has to be followed by selection, from all the technical possibilities, of an appropriate solution.

Although a systems approach is advocated, the book is mainly based on an examination of food chains—linear linkages without feedbacks. The editors recognise this but do not greatly emphasise that, when governments are recommended to focus on particular

inefficiencies, they should do so with due regard to all the consequences. The difficulties of doing this ought to be amongst the reasons for the modelling activity recommended.

It will certainly be necessary, as is strongly argued, to aim at high yields *without* high inputs of support energy, but this may not be sufficient. No-one would quarrel with the conclusion that "the greatest potential improvement thus lies in the area of conventional agricultural technology", but this is always likely to be so in the short term (if we mean by improvement greater food production). But if much more food was grown and harvested, the dilemma would remain, that the hungry people are also desperately poor.

Despite these criticisms, this volume is a constructive step in the right direction and will be of great value to all those interested in the application of technology to efficient food production.

C. R. W. Spedding is Professor of Agricultural Systems and Head of the Department of Agriculture and Horticulture at the University of Reading, UK.

Foresight and responsibility in land use

John Andrews

The Thames Transformed: London's River and Its Waterfowl. By J. Harrison and P. Grant. Photographs by P. Harrison. Pp. 240 (André Deutsch: London, 1976.) £5.95.

INDUSTRIALISED British estuaries have recently begun to receive the attention necessary to retain or restore their natural productivity. The Thames exemplifies the work, its results and the complex problems to be solved if the international importance of wildlife in our major estuaries is to be maintained.

One-third of *The Thames Transformed* deals with the inner estuary, describing well the decline of water quality to the point where the river was a deoxygenated broth in which neither bird, fish nor weed could persist, and outlining the remarkable work of the London County Council/Greater London Council and the Port of London Authority in restoring reasonable conditions in which wildlife could return. Ironically, continued improvement has now reduced the massive *Tubifex* population on which the wildfowl feed, so that their numbers are declining, whereas waders have lost

feeding areas due to reclamation.

The authors are experienced amateur ornithologists, Jeffery Harrison being known particularly for work on wildfowl and his concern for the north Kent marshes. Nearly half the book is devoted to this area—the Essex shore is dismissed in a cursory eighteen pages—and deals almost exclusively with wildfowl, including detailed historical accounts of occurrences. There is no discussion of the ninety-thousand waders or other bird populations, nor of the botanical, entomological and other important scientific features of the outer estuary. Concentration on the wildfowl produces recommendations about the agricultural management of the marshes which are not in the best interests of the total biological resource, whereas the conflict between wildfowl—particularly brent—and farmers, is glossed over. The identification of current industrial and recreational threats is valuable but too brief to demonstrate fully the complexity of the problems, both from the point of view of the 'developer' faced with real social and economic issues, and of the 'conservationist' striving to maintain scientific interest.

The authors suggest that the north Kent marshes should become a National Park rather than a National Nature Reserve, perhaps because the Nature Conservancy Council (NCC) has never had the finance required. Indeed, the money for research and land acquisition which resulted from the Maplin Airport threat, were gained for NCC

by the voluntary bodies which fought the Bill at Committee stage. It seems odd that there is no mention of this and some other important milestones, including the establishment of the NCC/Ministry of Defence Advisory Committee on Foulness and of the Thames Estuary Wildlife Conservation Group. The authors do give credit, however, to the conservation efforts of the PLA, the Central Electricity Generating

Board, and others. By encouraging them, their book can only help conservation which depends so much on the foresight and responsibility of all involved in land-use decisions. □

John Andrews is Head of the Conservation Planning Department of the Royal Society for the Protection of Birds, dealing with land-use issues affecting birds throughout the UK.

Baffling disease

C. H. Andrewes

Influenza: The Viruses and the Disease. By Sir Charles H. Stuart-Harris and G. C. Schild. Pp. x+242. (Arnold: London, 1976.) £12.

THE remarkable properties of the causative virus have made influenza a most fascinating yet baffling disease. Despite a vast amount of work there is still much to learn, particularly about how the infection can be controlled. Stuart-Harris and Schild's book is packed with information. For much of his working life, Sir Charles Stuart-Harris has been involved with the disease; he has in particular cultivated the area in which clinical and laboratory medicine overlap. Dr Schild was until recently in charge of the WHO World Influenza Centre at the National Institute for Medical Research, London, UK. Each of 12 chapters is followed by a list of references varying in number from 40 to over 100. Even these, as the preface points out, represent only a selection from the vast world literature.

We have first an outline of "the modern history of the study of the causes of human influenza". The authors pay only brief attention to work preceding the discovery, in 1933, of the susceptibility of ferrets to infection. Indeed there is only passing mention of Pfeiffer's so-called influenza bacillus, *Haemophilus influenzae*. Nor is much space devoted to outbreaks in earlier years, for the identity of the infecting agent of those times can only be inferred. Even the catastrophic 1918-19 pandemic is not considered at length.

Having reviewed the most important laboratory findings, including transmission to animals, cultivation and interference, chapter 2 discusses in great detail the molecular biology and replication of the virus. There are many excellent and well reproduced electron micrographs. These depict the whole virus and its separated components, and are supplemented by diagrams clearly explaining the structure and mode of replication of the virus.

Description of the viral antigens leads on to discussion of virus variation, the minor changes known as 'antigenic drift' and the periodical major 'antigenic shift' that herald pandemics. We learn how two viruses growing together in culture or in living animals may exhibit recombination of characters, resulting in viruses with hybrid properties.

Chapter 4 deals with the influenza viruses of swine, horses, birds and other species; for it is now recognised that man is only one host of a versatile family of viruses. There is increasing interest in the possibility that new pandemic strains may arise from recombination between human and animal viruses. The authors are, however, very cautious and write: "At the present time it seems somewhat premature to assess the significance of these findings . . .".

Chapter 5 describes the disease in man and its complications. We then have two chapters on epidemiology, with numerous diagrams illustrating the variations in mortality and morbidity from year to year. The authors admit to being puzzled as to where the virus goes between outbreaks and why older viruses disappear when new antigenic variants appear. There are full references to the work that led to the doctrine of "original antigenic sin". This concerns the remarkable fact that a person's first experience of a virus determines the pattern of his subsequent antibody responses: these are largely directed against the virus he first encountered rather than against more recent ones. When tests are made in the light of this, against various animal viruses, all sorts of intriguing questions arise about the nature of the influenza viruses involved in outbreaks of earlier years.

Studies of immunity, next described, deal largely with the relation between immunity and the level of antibodies in serum and in nasal secretions. Clearly it is vital to understand these matters if vaccination is to be attempted with any success. The results of several trials with inactivated vaccines of various kinds are reviewed in detail, and there is discussion about the best strategy for vaccine administration.

There are obvious theoretical advances

in the use of a live attenuated vaccine: it can be given as a spray and thus be much more acceptable; and it would be vastly cheaper. Methods used for virus attenuation are described together with the results of trials; those on a large scale have been carried out mainly in the USSR and Yugoslavia. It seems that, although acceptably attenuated vaccines are available, the results of trials have been variable. The authors suggest that live vaccines are more likely to be effective "when a new pandemic virus is triggering off a new cycle of epidemics". Otherwise, killed vaccines may be better.

"Some progress" is reported in the field of chemoprophylaxis and chemotherapy. Amantidine, in particular, is discussed, as is the theoretical basis of the ways in which drugs may contrive to upset replication or release of virus.

The final chapter provides details of the various techniques in use for the isolation and assay of virus, and of the various serological methods in use. Particularly useful will be the accounts of the recently described "single-radial-immunodiffusion" and "single-radial-haemolysis" tests.

A postscript discusses the recent excitement over the small outbreak due to a swine-like virus in New Jersey. As is wisely pointed out, "spread of swine influenza viruses from pig to man and their restricted spread in the population may have occurred from time to time in the past without detection".

The book will be of great value to workers in this field and to those concerned with preventive measures. It invites comparison with another, much smaller, popular book on the subject, *Influenza, the last great plague* by Professor W. I. B. Beveridge (Heinemann Educational: London, £2.95). Stuart-Harris and Schild's book is full of well documented facts, but the authors are very chary of even modest speculation. Not so, Professor Beveridge; he freely discusses pandemics from 1700 onwards, considers the birth of pandemics, and seems to have little doubt about the role of animal viruses in their genesis. Whereas one book describes the "trees" in most valuable detail, the other makes a bold attempt to discern the nature of the "wood".

The only serious criticism of Stuart-Harris and Schild's book is that the print is annoyingly smaller than usual; doubtless the price would otherwise be even higher. One hopes that the rising cost of books will not cause publishers to lower their standards of presentation.

Sir Christopher Andrewes was Deputy Director of the National Institute for Medical Research, London, UK, from 1952-61, during which time he was also in charge of the WHO World Influenza Centre.

Climatic pipe dreams

R. P. Pearce

The Cooling. By G. Ponte. Pp. 306. (Prentice-Hall: Englewood Cliffs, New Jersey, 1976.) \$8.95.

THE author of this book is described on the fly-leaf as having been an arms control researcher and who has since worked as a television and radio commentator. The book is about world climatology, its scientific as well as its wider human aspects. I, the reviewer, am a meteorologist who has spent a large part of his life in a University environment, attempting to solve some of the enormously difficult scientific problems which the atmosphere poses, of which those relating to longer-term climatic changes on the global scale are perhaps the most challenging of all.

I can only describe the experience of reading this book as having been something resembling a meteorological nightmare. Such a hostile instinctive reaction to a book makes it difficult for any reviewer to present what he feels he will regard in retrospect as a fair and reasoned appraisal, and I will therefore make every effort to give the author as much credit as it seems possible to give him, bearing in mind the highly controversial nature of the topic on which he has chosen to write.

The first part is an account of existing knowledge of climatic trends, particularly those of the past 20,000 yr or so, and of the physical factors determining climate. The presentation emphasises the role of solar radiation and discusses processes likely to result in variations of solar energy output. The second part examines the influence climatic factors have had on human activity from prehistoric to more recent times. The increasing susceptibility of the rapidly expanding and technologically developing human population to climatic variability is strongly emphasised; an appreciation of this by governments is illustrated by their involvement in discussions of schemes to control weather in various ways—for example, by hurricane modification, rain enhancement by cloud seeding, and the construction of barriers to modify ocean currents. Illustrations are also drawn, mainly

from the US experience in Vietnam, of the use of weather modification as a weapon of war. The severe consequences of adverse weather such as droughts and hailstorms on food production over large parts of the globe are discussed in some detail, and the activities of weather modification groups to alleviate the worst effects of these phenomena are given some prominence. The legal aspects of these activities are also discussed.

The third and final part of the book looks to the future. Taking as its basic proposition that there is a one-in-ten chance that the Earth's climate will cool and become more hostile to and seriously affect human activity over a large part of the globe within the next hundred years, it examines in some detail three possible options (apart from doing nothing) open to us for meeting this situation. The first is to adapt ourselves to the anticipated new conditions (for example, by introducing mandatory birth control to stabilise the world population, accompanied by food, water and energy rationing); the second is to try to modify climate locally—for example, by building a dam across the Straits of Gibraltar; and the third is to try to alter the global climate by, for example, blackening the polar ice caps or damming the Bering Straits. The author, after a brief reference to present international research efforts under the Global Atmospheric Research Program and stressing the urgent need for more research, concludes that the wisest course is a flexible mix of options.

The work is written in a highly sensationalist style which never lets up. The author's insatiable urge to impose his ideas on the reader and saturate him in his verbal imagery frequently leads him to use phrases which are logically absurd and amount to little more than scientific mumbo-jumbo. An example of this occurs in his description of a stationary high pressure area over Greenland as "causing a slight vacuum effect in wind patterns that increased cold winds sweeping down into Europe". Another is his description of tornadoes as "masses of cold air bumping into each other in thunderstorms". Some of his diagrams, too, are inaccurate. One of these shows the intertropical convergence zone as penetrating north of the Himalayas and up into Mongolia during the period 1900–1940!

But there is another aspect of this book which I find highly irritating and irresponsible. This is its almost total disregard and arrogant dismissal of the substantial progress made by the

worldwide scientific meteorological community in recent years in getting to grips with the fundamental problems concerning the physical basis of climate. The Global Atmospheric Research Program gets a mention here and there, but this hardly compares with the attention given, for instance, to the work of those who predict that an ice age could be upon us in seven years, or who talk of "punching holes" through the ozone layer with laser beams as a possible means of waging war on an enemy. The US National Academy of Sciences has recently produced a highly professional and valuable report (*Understanding Climatic Change: A Program for Action*) but this is referred to only briefly in an Appendix; and the Academy itself is cursorily dismissed as a "staid group".

All this is a great pity and, I fear, could even be dangerous. Lay individuals reading this book (including politicians, one of whom has contributed a foreword) could easily be misled into thinking that meteorologists know enough about global atmospheric behaviour to make a reasonable guess at what will happen to our climate over the next hundred years; and that we are on the verge of being able to change climate to provide a more favourable environment for mankind. The impression is given that only those meteorologists prepared to indulge in climatic prediction and weather modification have any real concern for applying their science to the benefit of humanity and that the others are just sitting back and taking little effective action.

It is a pity that the author has not directed his obvious talents as a publicist primarily towards drawing the attention of those who read his book to the great economic importance of weather and climatic research and to the need for strong financial support for the activities of those who are, and have been for many years, attempting to fill the large gaps in our scientific knowledge of the atmosphere and oceans, and the factors determining their behaviour, particularly on the longer time scales. Contrary to the impression conveyed by the author, most meteorologists see these as the primary goals of the science at this stage in its development. Until we have made more progress in this direction, reliable climatic prediction and control, the main themes of this book, are but pipe dreams. □

R. P. Pearce is Professor of Meteorology and Head of the Department of Geophysics at the University of Reading, UK.

British weather

H. H. Lamb

The Climate of the British Isles. Edited by T. J. Chandler and S. Gregory. Pp. xviii+390 (Longman; London and New York, 1976.) £5.95.

THIS useful book is likely to win wide acceptance as the fundamental reference work that its title implies. It presents a mine of information within a handy size and will serve many practical needs. Its editors, who are the heads of the Geography Departments of Manchester and Sheffield Universities, planned the book as a successor to E. G. Bilham's classic work which appeared under the same title in 1938: this new version, written with more awareness of climatic change, and incorporating a chapter on that topic, is to provide up-to-date observational data, including much (for example, on pollution) that was not available 40 years ago, and an introduction to modern ideas and analysis techniques (notably the greatly increased use of synoptic climatology). Although 12 of the 15 chapters are by scientists holding university appointments and only three by present or recently retired members of the UK Meteorological Office staff, the Office, together with the Irish Meteorological Service and their official publications, have provided most of the data.

The book is on about the same scale as Bilham's, having 390 pages to his 347 and 110 maps and 80 tables to his 101 and 63. Like all works made up of contributions from different authors, however, the style and standard of the chapters in the new work is somewhat uneven (although as regards most, not badly so). One is not told about the qualifications of the authors, but the research fields preferred by all but two of them can be recognised in their works cited in the 560-item bibliography (a very good list of modern references, although omitting some of C. E. P. Brooks's works, such as his *Climate in Everyday Life* and *British Floods and Droughts*, which are still useful).

It is sad to have to criticise anything in such a useful straightforward job as this compendious book. Yet the text is presented in a thin spider's web of print which is also too large for the spacing of the lines and fades to an indecipherable greying of the page in poor light in which Bilham's older text is still clearly readable. Another unhappy choice is the bold poster-print of

page-numbers in the index which has no point in combination with the spidery print of the subjects to be identified. The style in itself is not unattractive but is more appropriate to a mail-order catalogue than to a book that is to be read and re-read carefully over many years. By contrast, the printing of the many maps and tables is mostly good; but it is too small, so that a magnifying glass is needed to check many details. In some cases this, combined with awkward spacing of the figuring (as in Fig. 6.6 on p141), presents a real danger of misreading 1.0 as 10 and 2.5 as 25, and so on. It is odd, too, that so few publishers seem to realise the virtue of giving the key date—the year(s) and/or month or season—of each map of a series boldly on the map rather than in a list in small print in the group caption. There are also a few complaints about language. The Gaelic word *Eire*, which means the whole island of Ireland, is wrongly used in the title of the Irish Meteorological Service and elsewhere in the book to relate to just the 26 counties of the Republic. Another author prefers to use the five-syllable word "altitudinal" when he means "height" differences. In these changing times the fashion for changing names presents problems to writers who have to refer to the geography. This book boldly uses names of new counties in England and Wales, possibly before it is certain that they will stick.

In writing of the book's many virtues it is hard to know what to stress. This reviewer will probably find most occasion to refer to the data in the chapters on wind, radiation, temperature, precipitation, the climates of coasts and inland water bodies, of soils, slopes and vegetation, and the climates of towns. But nearly every chapter is packed with data, presented in forms likely to be of wide usefulness, and some of the other chapters also (for example, on synoptic climatology) are very good and up-to-date.

A few samples of items that struck one reader as interesting either *per se* or as indicating lines of development of the subject will illustrate the content and flavour of the book. First, a point that indicates *inter alia* where further development is needed: the presentation (pp62–68) in text, map and tables of extreme windspeeds having average recurrence periods of just once in 50 and once in 100 yr, derived by sound statistical theory and adopted by the British Standards Institution for basic design windspeeds for buildings, are based in many cases on only 20–30 yr of record (and, in at least some cases, it may be presumed based on the

very unrepresentative decades earlier in this century). The table of average yearly number of days with gale at 19 sample places illustrates not only the real gross geography of gales in these islands but how localities (and other things) may change with time and affect the issue: The second lowest frequency of gales (1.3 days per year) is at Mildenhall, Suffolk, now on the edge of Thetford Forest, an area which was known in the seventeenth century when it was open barren land for its storms of blowing sand that ruined many estates.

It is odd that there is no reference to hail in the index, but in the precipitation chapter we learn (p129) that this is an item which has been insufficiently studied and for which data are sparse. Many readers will be interested in the opportunity to compare the raininess of different places in terms of the average duration of rainfall: within the British Isles (in the 1950s) this ranged from 407 h yr⁻¹ at Ipswich (427 in the London area) to 616 at Plymouth, 1,157 at Swansea and up to 1,422 in the Western Highlands of Scotland. Cloud-cover apparently varies much less widely, from an overall average of 6.7 tenths at Aberdeen and 7.0 in London and East Anglia to 7.8 in the west and north of Scotland. Another aspect of the western climates comes out in the temperature chapter, where the extreme south-west, the coasts of Wales and south-west Ireland, so favoured for retirement, are pointed up as the areas where the average winter temperatures are not low enough for the vegetation (and the grass of your lawn!) to cease to grow.

Other interesting features include a map of the summer albedo of England and Wales, and diagrams detailing the radiation falling hour by hour on slopes of various angles facing north, east and south. A map of the frequencies of fog, with the greatest frequencies (up to 60–70%) occurring on the high ground, reminds us how poorly represented are the hills in this country by observing sites since the discontinuation already in 1904 of the pioneer Ben Nevis Observatory. There is much that is interesting also in the chapter on coasts and inland waters, although we learn that most of the study of the climate of the latter so far has been left to the freshwater biologists.

This book is a worthy successor to Bilham's, keeping abreast of the climate itself and our developing knowledge of it and its impacts. Altogether good value at the price. □

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Engineering geology

J. L. Knill

Principles of Engineering Geology. By P. B. Attewell and I. W. Farmer. Pp. xxx+1045. (Chapman and Hall: London; Halsted/Wiley: New York, 1976.) £25.

ENGINEERING GEOLOGY has been the main growth subject within the industrially-orientated aspects of the Earth sciences during the past two decades. The question remains, however, as to whether the success of engineering geology has been a consequence of the development of a new discipline, or is more fairly a reflection of the vocational opportunities for geologists within the construction industry.

The origins of engineering geology, as it is now defined, lay in the heavy construction industry in the early years of this century when geologists were invited to advise firstly on ground problems encountered during construction, and subsequently to assist in the prediction of difficulties during the earlier phases of investigation and design. At that time soil and rock mechanics did not exist as such and so it was natural in civil engineering for geologists to continue to be employed in the United States for the following decades; it was not until the 1950s that systematic education and growth in employment opportunities began to develop on a world-wide scale.

Soil mechanics was, by then, a well-established branch of engineering science, and subjects such as rock mechanics and hydrogeology were also firmly established. Perhaps it was inevitable that engineering geology has tended to move into a central position within the engineering earth sciences, attempting to link together the interactions between rock, soil and water as engineering materials within the realities of natural geological conditions and engineering problem-solving during investigation, design and construction.

There is, in consequence, a considerable element of choice not only in the balance between the component subject areas but also in the extent to which these subject areas are integrated with one another. On the one hand, it can be argued that the professional engineering geologist is essentially a geologist and his knowledge of the engineering disciplines should be maintained at a level that enables him to be able to communicate effectively in an engineering environment. On the other hand, engineering geology can be viewed as representing a balanced approach towards geotechnical engineering drawing together soil and rock mechanics, hydrogeology and geology into a unified whole

to that the engineering geologist adopts the role of an engineer.

The authors of this textbook have been concerned for some years with the organisation of a postgraduate course in engineering geology at Durham University, and the structure of the book reflects both the philosophy of this course, and the associated research activities. The book falls into two broad sections, the earlier part providing an approach towards the study of the engineering properties of engineering soils and rocks, whereas the later part deals with more practical, engineering-orientated topics.

The opening chapter provides a general coverage of minerals and rocks, drawing particular attention to clay minerals and their identification and this is followed by a concise review of soil mechanics. The succeeding chapter is a major treatment of the geotechnical and geological characteristics of clays and clay shales, much of the relevant material having been drawn from unpublished research. The text turns to rock as a material, and the influence of symmetry and preferred orientation of micro- and macrofabrics on the properties of rocks and soils. The progressive approach of the authors towards the development of macroscopic fabric diagrams related to stability/instability fields is of particular interest.

At this point the book seems to change step, turning systematically to site investigation, groundwater, soil and rock slope stability, techniques of ground improve-

ment, and the assessment and control of water resources. Most surprisingly, the application of geology to underground works and foundations, two important facets of the subject, are almost completely ignored.

This textbook is an interesting view of engineering geology, more strongly reflecting the engineering aspects than the geological. The arrangement of the book into two parts with somewhat different themes, is, however, not wholly satisfying nor has there been adequate control of excessive detail which penetrates into the text. For a book in excess of a thousand pages and at a price that will deter many, more careful structuring of the book with regard to the potential market could have yielded a shorter, cheaper and more comprehensive text.

The publishers suggest that the book will be of value for courses in both civil engineering and geology; at this price it can only possibly attract those who have already chosen engineering geology or geotechnical engineering as their career field. It has also been suggested by the publishers that "the book will itself eventually become synonymous with the subject area of engineering geology". Many will debate this prediction but it does not detract from the valuable service that the authors have done in drawing together so much useful material. □

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Dating in archaeology

Elizabeth Slater

Dating in Archaeology: A Guide to Scientific Techniques. By S. Fleming. Pp. 272. (Dent: London, 1976.) £8.95.

A DECADE AGO, when the application of scientific dating techniques to archaeological material was gaining momentum, this book would have been a welcome addition to the bookshelves of archaeologists. Non-specialists then needed a general introduction to the available methods, with some indication of their potential and limitation, to illustrate the wealth of information that could be extracted from archaeological material and to assist them in the assessment of the validity of the dates produced.

This book follows a standard format with sections devoted to the main dating methods, giving information on the method, the materials to which it can be applied, its accuracy, associated

problems and selected examples of its application. There are eight main chapters, plus a useful introductory chapter on the chronological patterns that were developed before the main impact of scientific dating methods, and a final chapter on scientific techniques applied to art history. The methods which reveal aspects of climatic changes are considered in an appendix and the other appendix contains an outline of the pre-dose thermoluminescence technique.

One of the greatest problems facing archaeologists is the interpretation of the information yielded by scientific dating methods. They are left with dates, plus associated errors, and a bewildering array of methods for the manipulation of the data. This problem is more readily acknowledged in the case of radiocarbon dating but it is inherent in all dating methods. The author gives no guidance in this matter, nor does he mention all possible procedures. He may feel that it would be premature to do so, and, as he says, "So many of the finer details of radiocarbon variations remain unresolved that no strong attempt has yet been made to routinely correct older conventional dates...". But he adds: "the

reader may be tempted to adopt at least one form of correction factor to produce more absolute ages..." (p73). The non-specialist, who has to make a selection and evaluate conclusions, understandably requires assistance, or positive or negative recommendations. In the examples given of the application of the methods, Dr Fleming concentrates on the results, and not the validity of their interpretation.

The selection of examples of applications is wide-ranging, and one of the merits of the book is that it draws on material not readily accessible, but some omissions are rather surprising from an archaeological viewpoint. For example, scientific dating has had a considerable impact on the chronology of prehistoric Europe, but two references to the same paper is scant recognition of the lengthy debate which has produced a book and numerous papers. It may be considered that no conclusions are yet possible or that the topic has already been fully discussed, but readers familiar with the arguments will also be conversant with much work in this book. Also, the chronological framework discussed in chapter 1, and that indirectly derived from it, have been discussed in the light of new dating evidence, and a final summary of the continuing validity of the original patterns would have been helpful.

The topics discussed are continually being developed and modified, and the author has made a conscious effort to keep the bibliography up-to-date. Although it is useful to find descriptions of methods, like acid racemisation, which have so far only achieved limited recognition, from the point of view of archaeologists the changes in the main methods have been evolutionary rather than revolutionary in the few years to elapse since the last synopsis was published.

Despite the comments above, there is much of merit in this book. It contains a wealth of information, it is up-to-date, the methods are described clearly with an adequate amount of detail, and the diagrams are particularly clear. One distinctive feature is that analytical techniques and applications which have chronological implications are rightly included. It can certainly be recommended as the most up-to-date text for non-specialists, and the author is only unfortunate that he has followed a path which a number have trod. Perhaps the definitive book on dating, which Dr Fleming is admirably qualified to produce, is not destined to appear in the immediate future. □

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History of the Nile valley

Gudrun Corvinus

Prehistory of the Nile Valley. By F. Wendorf and R. Schild. Pp. xxiv+404. (Academic: New York and London, 1976.) \$25; £13.75.

THE INTENTION of this book is to assemble and present all the available data and observations made during three seasons of fieldwork (1967-69) along the Nile in Egypt. The first part of the book covers in considerable detail the geology and stratigraphy of the Final Pleistocene Nile sediments between the Assuan and Fayum Depressions, including comprehensive descriptions of sites and sections. The second part describes and revises the sequence of stratigraphical and chronological events, and attributes the archaeological sites to cultural units.

The book is well illustrated with maps, section drawings, drawings of artefacts and photographs which clarify the rather lengthy descriptions of the sites. Regrettably a few names of the areas discussed are not mentioned in any of the maps.

The title of the book is somewhat misleading in view of the detail concerning the geology and palaeoenvironment; this latter is, nevertheless, of considerable value. The purely archaeological descriptions occupy only a small portion of the book.

Lake Rudolf Basin

G. E. Kennedy

Earliest Man and Environments in the Lake Rudolf Basin: Stratigraphy, Paleocology and Evolution. Edited by Y. Coppens, F. Howell, I. Clark, L. Glynn and R. E. F. Leakey. Pp. xxi+615. (University of Chicago: Chicago and London, 1976.) Cloth £11.90; paper £5.80.

PALAEANTHROPOLOGISTS have often discussed the value of an interdisciplinary, 'synthetic' methodology and yet this approach has not been highly visible in the literature. This book, and the recently published *After the Australopithecines* (ed. K. W. Butzer and G. L. Isaac; Mouton: The Hague and Paris; Aldine: Chicago; for review, see *Nature*, 262, 331, 1976) may represent a strong sign that broad interdisciplinary analyses of early man sites will become the rule rather than a

The most significant contribution that the book makes is the revision of the sequence of events of the Final Pleistocene and Early Recent periods. The authors have assembled and interpreted a considerable amount of new data, filling gaps in the existing knowledge of the prehistory of the Nile and leading them to revise previous concepts.

The principal amendments concern the two large aggradations of the modern Nile. The Ballana Mamas episode is regarded here as one unit of simultaneous dune formation and Nile silt aggradation. A number of carbon-14 dates place this period between 17,000 and 15,000 BC, two different archaeological complexes having been recognised (the Idfuian and the Fakhurian). Furthermore, the Darau Member of the Kom Ombo area and the late Sahaba Formation of Sudanese Nubia belong, according to the authors, to the same episode of Nile aggradation and are termed here the Sahaba-Darau aggradation. The archaeological sites attributed to this period, dated from 12,000 to 9,700 BC, are the Afian, the Sebilian and the Isnan industries.

The authors are to be commended in attempting to compile and interpret such a vast amount of data. The book will serve as an excellent base for further research and as a reference for comparative studies, and will mainly interest the specialist in this field. □

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rarity. It is no doubt a sign of this trend that reports of the hominids found at East Rudolf comprise only five papers out of a total of fifty in this volume. These papers, presented at a workshop held in Nairobi in September, 1973, have been arranged into three sections: Geology and Geochronology; Paleontology and Paleoecology; and Paleoanthropology.

From this volume it is apparent that there are areas of both agreement and disagreement among the thirty-eight scientists who were involved in the Nairobi workshop. An interesting area of agreement concerns the occurrence of a climatic change between 2.5 and 2.0 Myr BP. This change, which resulted in drier conditions and an expansion of grassland areas in the Rudolf basin, has been indicated by studies on pollen (Bonnefille), bovids (Gentry) and micromammals (Jaeger and Wesselman). Documentation of climatic change at this time is of particular interest because the first clear evidence of archaeological materials also occurs within this period. Although the relationship, if any, between climatic

change and the appearance of cultural materials is unclear, this interesting association requires more investigation.

From the standpoint of early man studies, one of the major outstanding questions is the date of the KBS tuff. The date of this tuff is crucial because there is good evidence of the presence of the genus *Homo* below this level. The results of radiometric studies have been contradictory, ranging from 1.6 to 2.6 Myr BP for this tuff. As Cooke's paper indicates, the earlier date for the KBS tuff may be suspect on palaeontological grounds. Cooke's studies on fossil pigs has shown that tooth size in one taxon, *Mesochorus limnetes*, found below the KBS tuff at East Rudolf most closely resembles that in specimens from the

nearby Omo area dated to 2 Myr BP.

This important volume contains a wealth of detailed and important information, and will be very useful at the professional level. Its information, however, will be less accessible to students. Although each of the three major sections is prefaced with an introduction, these are far too brief to really open the major issues and questions posed by each area of research. Such interdisciplinary studies are most valuable when they result in a synthesised and comprehensive view. Although such synthesis is missing here it is a minor defect in an otherwise fine volume. □

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Hominid and the bathwater

W. W. Bishop

Geology of the Olduvai Gorge: A Study of Sedimentation in a Semi-arid Basin. By R. L. Hay. Pp. xvi + 203. (University of California: Berkeley, Los Angeles and London, 1976.) £15.75.

RECENT interest in Earth science has promoted fruitful growth of such 'interface' studies as geochemistry, geophysics, and geobiology. It comes as no surprise to see the common ground between geology and archaeology also attracting attention. What is unusual is to find, within the 200 pages of a single-author volume, a blue-print for the application of various disciplines to the study of sediments yielding hominids and artefacts. These include stratigraphy, geochronology, sedimentary petrography, mineralogy, isotope geochemistry and amino acid chemistry, to mention but a few.

On this substratum Dr Hay builds a sophisticated time framework for the past 2 Myr and reconstructs palaeoenvironmental settings for the hominids, their artefacts and, by inference, their activities in the changing palaeogeography of the Olduvai region.

Olduvai was the area where large scale excavations by Louis and Mary Leakey showed geologists, vertebrate palaeontologists and even some archaeologists, how local microstratigraphy could be studied to set early hominids and their palaeolithic tool kits against a factual background from which to decipher the environment of burial and, with luck, the habitat of life. Dr Hay, with early experience of the stratigraphical complexity posed by arid intermontane basins in western USA, was the ideal successor to other geologists (Hans Reck, E. J.

Wayland, P. J. H. Boswell, P. E. Kent and R. A. Pickering) who worked at Olduvai. It was fortunate that he appeared on the scene in 1962 after initial over-concern with alleged pluvial-interpluvial 'climatic stratigraphy' had been passed over in favour of a return to 'normal' stratigraphical and geological methods.

The finding of the skull of *Australopithecus* (*Zinjanthropus*) *boisei* in 1959 was the trigger mechanism for new investigations. Olduvai witnessed the first application of K-Ar dating to rocks as young as 1 or 2 Myr. Dr Hay tabulates the 57 K-Ar ages established for Bed I since the initial investigation by Curtis and Evernden, and discusses them in relation to fission-track dating and palaeomagnetic polarity data. Beds II to IV yield few reliable K-Ar dates, but numerous radiocarbon ages are given spanning the past 30,000 yr at Olduvai.

For the archaeologist the most important sections of the book are those presenting palaeogeographic reconstructions, both descriptive and visual (18 out of 27 maps in a total of 63 text figures), based upon detailed stratigraphical columns, which are also excellently illustrated by pull-out sections. These provide the factual and the interpretative environmental record against which Dr Hay records the hominid and artefact content of each formation. The final chapters present a synthesis in the form of a four-page evolutionary history of the Olduvai basin and a discussion which in 7 pages distils evidence for the pattern of hominid activities.

Despite its high price this is a book to purchase as well as to read, for it seems certain to become a 'classic'. It demonstrates how geochronology and the study of palaeoenvironments may be used to obtain full value out of both the fossil baby and the sedimentary bathwater.

W. W. Bishop was Professor of Geology at Queen Mary College, University of London, UK. He died on 19 February this year at the age of 46.

THE BULLETIN OF THE BRITISH MUSEUM (NATURAL HISTORY)

Unlike the usual research journal the Bulletins of the British Museum (Natural History) appear at irregular intervals and each is a comprehensive monograph on a specific subject.

IMPORTANT TITLES FOR THIS SPRING

THE OSTEOLOGY OF *NOTELOPS* WOODWARD, *RHACOLEPIS* AGASSIZ AND *PACHYRHIZODUS* DIXON (PISCES: TELEOSTEI)

P. L. Forey
Bull. B.M.(N.H.) Geology series
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1. INFRAGENERIC CLASSIFICATION

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Development of bioenergetics

A. R. Crofts

A New Look at Mechanisms in Bioenergetics. By E. Racker. Pp. xiv+197. (Academic: New York, San Francisco and London, 1976.) \$9.50; £5.80.

PROFESSOR RACKER has ranged widely in his experience of research in bioenergetics, and this small book chronicles his activities in the field over the past ten years or so. The book takes the form of, and is based on, a series of lectures to students; it is aimed at the undergraduate or early post-graduate level rather than at established research workers. The chapters are prefaced by some admirably chosen quotations, and the text is interspersed with homilies and cautionary tales. The subject matter covered includes substrate level and electron transport-linked phosphorylation, ATP-driven ion pumps, resolution and reconstitution of the different complexes of enzymes which catalyse these activities, and details of the enzymology of these complexes.

Racker has played a major role in catalysing an acceptance of the chemiosmotic view of the mechanism of catalysing an acceptance of the chemiosmotic view of the mechanism of coupling between electron transport and phosphorylation. The book describes fully the very nice work carried out by Kagawa, Hinkle, Kandrach, Ragan and many other colleagues in collaboration with Racker, on the reconstitution of oxidative phosphorylation in liposome vesicles, using the purified enzyme complexes of the mitochondrial apparatus. This work had a strong biochemical appeal, which won over many of those in the field (and there were many) who had blanched at the large body of biophysical evidence previously available, that supported a similar view. The demonstrations that the mitochondrial ATPase, cytochrome oxidase and NADH₂ dehydrogenase could separately act as proton pumps when incorporated into liposome vesicles, and that when the ATPase was incorporated together with any of the electron transport complexes, the electron transport could drive phosphorylation, provided a powerful support for the argument for a chemiosmotic mechanism.

Most dramatic of all, however, was the demonstration, in work with Stoeckenius, that the purple membrane protein of *Halobacter* could be similarly reconstituted as a light-driven

proton pump, and that inclusion of the ATPase in the vesicles led to a preparation capable of photophosphorylation. In this case, a clear distinction was evident between the requirement for a device for pumping protons, and an electron transport system. The former was necessary, but not the latter, so that the obligatory coupling between electron transport and phosphorylation predicted by chemical and conformational hypotheses for the mechanism of coupling could be excluded. It is perhaps a minor criticism of this book that the controversy over the mechanism of coupling is framed in such a way as to highlight these biochemical contributions. This is only human; the book cannot be recommended as a completely balanced view of the development of the subject, but, rather, as a personal view.

The other criticism I have to make relates to the "lessons" which crop up here and there, especially at the beginning of the book. These are asides, included perhaps to entertain the reader, and for the most part they are sensible and amusing. Some of them, however, strike me as ham-fisted. For example, Racker is being both cynical and unjust

when he teaches with reference to Mitchell's preliminary paper on chemiosmotic coupling: "If you propose a brilliant, new hypothesis and avoid being specific, you probably will be ignored. If you want attention be sure to include a few features that are not essential for the main theme, but open to attack". Several other of the lessons also read like tips to a trainee con-man rather than distillations of wit and wisdom. The quotation above reflects more Racker's own late conversion to Mitchell's point of view than the considerable impact of this early paper; Racker (to his credit) draws attention to this tardiness of his in the preface.

These criticisms are, however, trivial, and I would not wish to discourage anyone from reading this book. It provides a lively and fresh insight into the subject, avoids obscurity and mystification, and provides a more than usually straightforward account of the development of an area of research, mistakes and all, in one of the most influential laboratories in the field. □

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Molecules in solution

J. M. Creeth

Biological Macromolecules and Polyelectrolytes in Solution. By Henryk Eisenberg. Pp. xviii+269. (Clarendon: Oxford; Oxford University: London, 1976.) £16.

Few developments in physical biochemistry over the past two decades have had greater impact than that initiated by Casassa and Eisenberg in the years 1960-64. In a notable series of papers, a theory was described which dramatically simplified the measurement of molecular weight in ternary systems. This was most timely: it coincided with a surge of interest in charged polymers and with the recognition of the subunit structure of many proteins, where measurements were often necessarily made in high concentrations of urea, etc. Such systems were now shown to be amenable to analysis by pseudo-ideal equations, with the twin provisos that the solutions were at dialysis equilibrium and, most characteristically, that the multiplying coefficients (for example, a specific density increment) were defined at constant chemical potentials of the permeating components. In this

way, all the effects of interaction (no matter how great) disappeared with the introduction of the appropriate coefficient.

The theory was very soon accepted, but, by the majority, perhaps, more on trust than by conviction, for the mathematics were undeniably tedious. The appearance of a volume by Professor Eisenberg devoted to a comprehensive account of macromolecules in solution is therefore an important occasion. The author has taken full advantage of the opportunities presented to him: a useful summary of the chief structural features of proteins and nucleic acids is followed directly by chapters on the fundamental thermodynamics of ternary solutions. Here the Casassa-Eisenberg concept is developed from first principles: this section contains a clear exposition of the meaning of the preferential interaction parameter, which should be read carefully by anyone tempted to refer loosely to 'solvation' or 'salt-binding'. The notation has been considerably simplified, and accordingly the essential concepts can now be grasped much more readily.

In the chapter devoted to partial volumes, density and refraction increments, the significance of the restriction of constant chemical potential is fully explained: a simple sketch brings home forcibly the meaning of each step. Here also we find the essential conclusions concerning the magnitude of the interaction coefficients: the practical demonstration that these do not

vanish in the limit of zero concentration is, of course, the justification of the method. Most readers, I think, would now agree that the conventional 'buoyancy factor' in the equations of sedimentation should be replaced by the density increment, although some would, perhaps, regret the loss of the conceptual aid provided by v , with its reminder that the experiment determines the molar volume.

The remainder of the book is devoted to the four chief experimental procedures—light scattering, sedimentation equilibrium, sedimentation velocity/diffusion, and viscosity—each being treated in terms of the new definitions when appropriate. In addition to conventional methods, the newer technique of intensity-fluctuation spectroscopy is described, and the chapter on sedimentation equilibrium includes a section on density gradients which is notable for its vindication of the original theory of Vinograd and colleagues for the distribution of charged macromolecules. Those wishing to find their way through the tortuous paths of associating systems will find the rather sweeping assumptions made in this field clearly set out and assessed, and a useful summary is given of the procedures devised by Yphantis and Roark for the elimination of the effects of non-ideality.

The equations of transport are distinctly more complex than those relating to equilibrium, but this section will interest many for its revision of the theory of Alexandrowicz and Daniel, and the subsequent proof that the limiting sedimentation coefficient of a charged polymer may also be described in terms of the macromolecular component alone, when this is formulated in terms of the theory.

The volume is completed by a series of appendices devoted to thermodynamic concepts (including irreversible process theory) and the references, which are conveniently divided into categories of broad interest and of specific detail. The general standard of presentation is excellent, the few printer's errors noticed being essentially trivial—no mean feat in view of the complexity of notation.

The book will undoubtedly please the theoretician, for the logic and elegance of the mathematics; however, I think that most of all, it will be a delight to the concerned experimentalist, who will find the essential practical procedures and necessary precautions summarised concisely, in readily accessible form, together with the underlying theory. No laboratory working with macromolecules in solution should be without its copy. □

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Darwinism at a cellular level

Leslie Brent

Immunology, Aging and Cancer: Medical Aspects of Mutation and Selection. By F. M. Burnet. Pp. 162. (Freeman: San Francisco, 1976.) Hardcover \$9.95; softcover \$6.50.

THIS slender volume is based on lectures given by Sir Macfarlane Burnet in 1975 to students at the University of California, Irvine, and at Southampton University Medical School. It is an audacious and successful attempt to scan the wider horizons of immunobiology and genetics by examining and linking such fundamental issues as immunological homeostasis, the ability of cells to repair errors created in their DNA during the mutagenic process, the problem of aging, the pathogenesis of certain genetic and autoimmune diseases, and cancer.

According to Burnet, the common thread of the book is Darwinism at a cellular level. Although other theories have been put forward, it is somatic mutation that is most likely to account for the great diversity of antibodies and the cells that produce or carry them, and it is somatic mutation—coupled with a diminishing effectiveness of DNA polymerases and other enzymes in aging organisms to repair DNA errors—that leads to the manifestations of old age. Likewise, it is the accumulation of DNA errors in lymphoid cells that results in autoimmune disease, whereas the same phenomenon in somatic cells in general leads to the formation of malignant clones that, clinically, we call cancer.

Burnet is as adventurous and vigorous as ever in his discussion of these problems. Although it is true that many of his ideas have been developed at greater length in his earlier writings, this does not in the least detract from the value of this book; on the contrary, it is immensely readable precisely because of the economy of argument. The combination of brevity and global approach nevertheless imposes its own limitations, examples and data are selected fastidiously and, despite occasional warnings that the author's notions may eventually be shown to be wrong, the reader is sometimes left in the dark about points that might have been regarded as detrimental to them. For example, the superficially attractive idea that mast cells are fully differentiated

T lymphocytes, bearing roughly the same relationship to each other as plasma cells do to B lymphocytes, is itself based on a questionable premise—that the immunoglobulin receptors of T cells are secondarily acquired from B cells shedding superfluous monomeric IgM. The existence of immunoglobulin-like T-cell receptors, let alone their origin, is still disputed. If it is true that T cells do not possess home-made receptors and that B-cell receptors are created by somatic mutation, it is reasonable to ask how it is that T cells have eluded this basic genetic mechanism and why it is that these cytophilic receptors are so difficult to demonstrate on T cells.

In the chapters on mutation and aging, Burnet explains that both bacteria and mammalian fibroblasts can make good errors in their DNA caused by *in vitro* exposure to ionising radiation. He proposes that the differences in the average lifespans of mammalian species can be largely, if not wholly, explained by differences in the efficiency of the DNA repair mechanism. Apart from the fact that this hypothesis disregards other possible factors, such as the more severe metabolic requirements imposed on small mammals by their high surface/volume ratio, even experts in this field are not yet certain of the extent to which DNA repair influences lifespan. In the words of R. E. Hart (in *Aging Carcinogenesis and Radiation Biology*, ed. K. C. Smith, Plenum: New York, 1976.): "Indeed, at this time it would be premature to infer that DNA repair in and of itself is a primary determinant of lifespan, much less to state that a single repair process is of more significance than any other". Be that as it may, Burnet does not envisage that immunological processes play a primary part in aging but, rather, that they are brought into play as a result of accumulated errors in aging somatic cells, thus producing autoimmune diseases; and that aging of the lymphoid cells themselves leads to decreased efficiency in immune surveillance, thus permitting the development of malignant clones. Little credence is given to the suggestion that viruses may be playing a critical role in some so-called autoimmune diseases, and in re-stating the hypothesis of immune surveillance there is no mention of the experimental evidence that has, in recent years, somewhat weakened this highly attractive idea—certainly so far as T-cell surveillance is concerned. I am thinking here mainly of the observations that neither "nude" (T-cell deficient) mice, nor mice treated from birth with anti-lymphocyte serum, show a markedly increased incidence of various kinds

of tumours. That immunosuppressed kidney allograft recipients have a greatly increased incidence of lymphoid tumours provides far from convincing evidence for the hypothesis, as the immunosuppressive drugs used could themselves be carcinogenic for lymphocytes or, alternatively, chronic stimulation by alloantigens might act as a trigger for malignancy in lymphoid tissues.

RNA polymerase

Walter F. Mangel

RNA Polymerase. Edited by R. Losick and M. Chamberlin. Pp. ix+899. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1976.) \$38.

GENE EXPRESSION and its control has, for the past 30 years, been one of the more fruitful areas of research for molecular biologists. The intriguing results of the bacterial geneticists enticed many biochemists and physicists to seek molecular explanations for such widely diverse phenomena as the inducible metabolism of sugars, the initiation of DNA synthesis and the temporal expression of bacteriophage genes. In most cases, they ended up studying transcription. This book summarises their progress and illustrates the multifarious and subtle ways that have evolved to control gene expression at the level of RNA polymerase activity. Now they are studying the primary sequence of *lac* operator DNA, the RNA primers for DNA replication and the phage-induced RNA polymerases.

Losick and Chamberlain are to be congratulated for having designed an excellent book which will be useful to those who desire a comprehensive introduction to the subject, as well as to those who are actively carrying out research in the field. It is also an excellent text for graduate students. The first half of the book contains an historical introduction by Weiss, an overview of the transcription process by Chamberlin, and eleven review articles that outline the active areas of research. The second half consists of 32 previously unpublished research articles. As with all Cold Spring Harbor monographs, this one contains an extensive and useful list of references.

In his introduction, Weiss describes how the discoveries of different but specific tRNAs and of nucleoside triphosphates as substrates for DNA synthesis led him to assay for an RNA polymerase activity in a crude rat liver

We must be grateful to Sir Macfarlane for giving us a highly readable and stimulating book, although the general reader should be warned that some background knowledge of immunology or genetics is desirable if he is to get the most out of it. □

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homogenate. And in the next article, Chamberlin summarises the work done in the intervening 17 years. His overview of both the molecular and catalytic properties of RNA polymerase is comprehensive, integrating properties of the *Escherichia coli* enzyme with those from other prokaryotic and eukaryotic cells. This is followed by a highly practical article by Burgess on the purification and physical properties of the enzyme. Zillig, Palm and Heil discuss the functions and re-assembly of the subunits, and Krakow, Rhodes and Jovin describe the use of inhibitors to study catalytic mechanisms. The intriguing problems of how RNA polymerase recognises a promoter, initiates transcription and terminates RNA synthesis at specific sites, are discussed in articles by Chamberlin, Gilbert and Roberts. Scaife delineates the progress in the location of the genes for RNA polymerase and the control of their expression. Transcription patterns in bacteriophage infections are controlled, in some cases, by regulatory subunits or bacteriophage-induced RNA polymerases, as outlined by Losick and Bautz. Roeder discusses the properties of nuclear RNA polymerases and some of the problems encountered in studying eukaryotic transcription. Finally, Kornberg defines the role of RNA primers in DNA replication.

Genetic evolution

Thomas H. Jukes

Molecular Evolution. Edited by Francisco J. Ayala. Pp. x+277. (Sinauer Associates: Sunderland, Massachusetts; Freeman: Reading, 1976.) Paperback £6.90.

THIS book is a compilation of thirteen papers that were presented at a symposium held at the University of California, Davis, in June, 1975. Most of the articles are more closely related to population genetics than to molecular evolution.

There is an introductory chapter by Francisco J. Ayala followed by chapters on genetic variation in natural populations (Robert K. Selander); genetic polymorphism and enzyme function (George B. Johnson); allozyme variation: its deter-

The research articles in the second half of the book are specific extensions of each of the areas described in the first half. There are three articles on promoter sequences. With nine promoters having been sequenced, common features, such as twofold axes of symmetry, are emerging. RNA polymerase will not, however, rebind to promoter fragments isolated by protection from DNase, so that additional elements remain to be discovered. The inhibitor, rifampicin, has been extremely useful in studying RNA chain initiation, and there are three articles which indicate that the mode of action of the drug is surprisingly complex. Rifampicin-resistant mutants made possible the mapping of the β subunit. In four articles, the locations of the other subunits are described and shown to be linked with the genes of some of the proteins of the translation apparatus. There are four papers on the regulatory subunits which become associated with RNA polymerase after bacteriophage infection. With the discovery that the rho gene maps at the *suA* locus, the termination step in RNA synthesis is a very active area of study and is represented by six papers.

Finally, there are some excellent articles on eukaryotic RNA polymerases from yeast, *Drosophila* and mammalian cells. Although the activity of RNA polymerase is modulated to control gene expression in prokaryotes, similar mechanisms have not yet been observed in eukaryotic cells. An elephant may still be a big *E. coli* but it is, ironically, less obvious when viewed at the level of transcription.

This is an excellent book on an important and fascinating subject. □

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minants in space and time (Michael Soulé); genetic strategies of adaptation (James W. Valentine); organismic and molecular aspects of species formation (Theodosius Dobzhansky); genetic differentiation during speciation (John C. Avise); biochemical consequences of speciation in plants (Leslie D. Gottlieb); protein sequences in phylogeny (Morris Goodman); molecular evolutionary clocks (Walter M. Fitch); evolution of genome size (Ralph Hinegardner); evolution of repetitive and non-repetitive DNA (Glenn A. Galau, M. E. Chamberlin, B. R. Hough, R. J. Britten and E. H. Davidson); and gene regulation in evolution (Allan C. Wilson).

The discussions of molecular matters occur principally in the final five chapters. Goodman's observations lead to his concluding that the evolution of globin sequences in vertebrates proceeds at different rates during different evolutionary periods. He attributes the "fast

evolution to positive natural selection for improvements in the function of hemoglobin and the slow evolution to stabilising natural selection after the improvements were fixed", following which "natural selection would then be able to shape the finer adaptations of the protein". It is possible to propose, however, various interpretations to account for such observations, and Goodman acknowledges this point.

Fitch, in discussing molecular evolutionary clocks, says that "we have several sloppy clocks", which may be an example of sloppy diction. He states that "the tRNAs are mostly paralogous", which was news to me, because there are many examples of homologous tRNAs for the same amino acid occurring in related species of organisms. Obviously, many thousands of such tRNAs have not yet been sequenced. Fitch tells us it came as something of a shock to him that Δ haemoglobin has never been sequenced, which leads me to comment that those who accept the sequences in the *Atlas of Protein Sequence and Structure*, instead of referring to the original literature from which the sequences are derived (sometimes by presuming the sequences of unordered peptides), should expect such shocks. Fitch gives a good discussion of the rates of 'silent' nucleotide substitutions. He challenges comparisons of snake, bird and turtle cytochromes *c* by suggesting that "either one or more of its sequences is incorrect", which is an easy out of the argument.

The review by Galau and co-workers is a valuable compilation of recent results on the rate of re-association of various types of DNA. Wilson presents an excellent summary of the differences in rates of evolution between frogs and placental mammals in support of his thesis that contrasts the rates of organismal change and structural gene evolution. The apparent similarities of different species of frogs to each other conceals the fact that they may be widely separated in terms of ancestral history, and also in terms of amino acid differences between their homologous proteins. Hinegardner discusses the wide variations in haploid DNA contents per cell in various groups of organisms. The DNA content per cell has increased during the history of life on Earth, but mammals do not lead the parade.

I wish that the term "nonsense" for polypeptide chain-terminating codons, and the non-word "missense" for mutations that produce single amino acid changes (as used and defined by Ayala) could be relegated to the graveyard of discarded and superseded neologisms. But new words never seem to die, or even fade away. □

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Somatic cell hybrids

R. T. Johnson

Cell Hybrids. By N. R. Ringertz and R. E. Savage. Pp. 366. (Academic: New York and London, 1976). \$29.50; £20.95.

THERE are relatively few events in the recent history of biology which have so fired and re-directed the imagination as somatic cell fusion. Suddenly, in the mid-1960s, the unexpected and unnatural seemed commonplace in cell biology. Somatic cells of diverse origin and nature could be brought together within a single cell, their interactions studied and their hybrid progeny cultured for subsequent analysis. The revolution that Sendai virus-promoted fusion brought to the embryonic subject of cell hybridisation not only permitted the achievement of a greater number of fusion events by a greater number of people but also generated the enthusiasm to develop hybrids between seemingly impossible combinations of cells. The search for agents that promote fusion between unlikely partners remains an area of continuing interest, with the recent demonstration of polyethylene glycol-induced fusion of human and plant cells.

The unusual attraction and usefulness of cell hybrids to biologists soon became evident from the growing number of publications covering a very wide area of contemporary biology. Cell fusion rapidly became one of the most attractive ways of detecting differences between cells. By combining two cells it is possible to monitor some of their differences by following interplay with respect to such general genetic activities as the initiation of RNA and DNA synthesis, and more specifically, to the extinction, re-expression and activation of single genes. Although fraught with difficulties, the analysis of the differentiated state by means of cell hybrids has now progressed to a stage where general rules for the expression of facultative functions can be expected before long. Similarly, the usefulness of somatic cell hybrids in gene complementation and mapping is in no doubt, and advances here and in the study of differentiated expression in hybrid cells are fortunate to have coincided with a revolution in methods for chromosome identification. Cell fusion has also provided an opportunity to study the nucleocytoplasmic interactions in mammalian cells in ways which are similar to the investigations so elegantly carried out using the technique of nuclear transplantation in large cells like Protozoa or amphibian oocytes.

In this well illustrated volume the authors have gathered together, for the first time, the accumulated work up to the end of 1975 on somatic cell fusion and cell hybrids. This is a large, complex, controversial and expanding field and we are therefore indebted to Drs Ringertz and Savage for this timely compilation and review of the subject, reduced to manageable proportions and written with clarity.

The aim of *Cell Hybrids* is to provide an up-to-date account of the contributions made by cell fusion and cell hybrid studies to major areas of somatic cell investigation including cell cycle control, the expression of differentiated functions and malignancy, and the mapping of constitutive genes. It is also intended that the volume should serve as an introduction to students and to workers in other areas. A useful background is therefore included in each chapter providing sufficient information for critical appraisal of results obtained.

Because of their limited lifespan animal cell heterokaryons are less frequently investigated than their hybrid progeny. As the authors stress, however, heterokaryons permit analysis of regulatory phenomena in the presence of complete genomes and should be included whenever possible in any full description of gene expression in hybrid cells which usually contain a reduced and occasionally modified complement of chromosomes. Possibly the most famous heterokaryons contain chick erythrocyte nuclei which, as Henry Harris showed 12 years ago, are reactivated from the quiescent state. Much of the elegant work on erythrocyte activation has emerged from the Karolinska Institute and this is well represented in *Cell Hybrids*.

The very great problems that attend the unravelling of genetic interplay in somatic cell hybrids may to some extent be reduced by the use of reconstructed cells, and a full description of this new area of cell manipulation is included. For example, the production of hybrids by the fusion of whole cells with microcells containing very small quantities of DNA not only predetermines the segregation pattern but may speed up and ultimately simplify the analysis of gene localisation and regulation.

The advent of cell hybridisation has permitted many new experimental approaches to major and seemingly intractable biological problems. A number of the problems have now been reduced in stature and others re-defined because of cell hybrids. We should be grateful to the authors for this comprehensive and readable work on the subject.

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articles

Interpretation of the Southern California uplift in terms of the dilatancy hypothesis

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The 25-cm uplift in Southern California is interpreted as being due to dilatancy of the crust, which started in 1961 along the San Andreas fault and spread laterally during 1964–69. The San Fernando earthquake of 1971 occurred in part of the dilatant volume, and is interpreted as a side-show of a future repetition of the 1857 San Andreas break, which had a magnitude > 8.

AN area of at least 12,000 km² was uplifted aseismically in the early 1960s in Southern California¹. The uplift was centered on the San Andreas Fault, where it reached a maximum of over 25 cm near Palmdale, 60 km north of Los Angeles. The portion of the San Andreas Fault that lies within the 15-cm uplift contour comprises the southern half of the last major earthquake in Southern California, the 1857 rupture, which had a length of about 350 km (ref. 2). The segment of the San Andreas Fault that broke in 1857 exhibited conspicuously low seismicity during the last 40 yr (ref. 3). This section is considered to be a mature seismic gap, which is expected to be closed by a large quake sometime in the future.

Castle *et al.*¹ offered two hypotheses to explain the portion of the aseismic uplift in the San Fernando area: (1) a north-south contractional strain effect coupled with creep on a deep part of the San Fernando Fault, or (2) a dilatancy effect. Thatcher⁴, elaborating on the first hypothesis, proposed that the north-dipping San Fernando Fault reaches a depth of about 50 km, cutting below the San Andreas and extending north of it, and he suggested that aseismic creep started at the deepest part of this thrust fault and propagated upwards, causing the uplift and culminating in the 1971 San Fernando earthquake.

We formulate here a dilatancy hypothesis to explain the observed uplift and to show that this alternative has some advantages that may make it more plausible than the creep hypothesis.

Uplift data

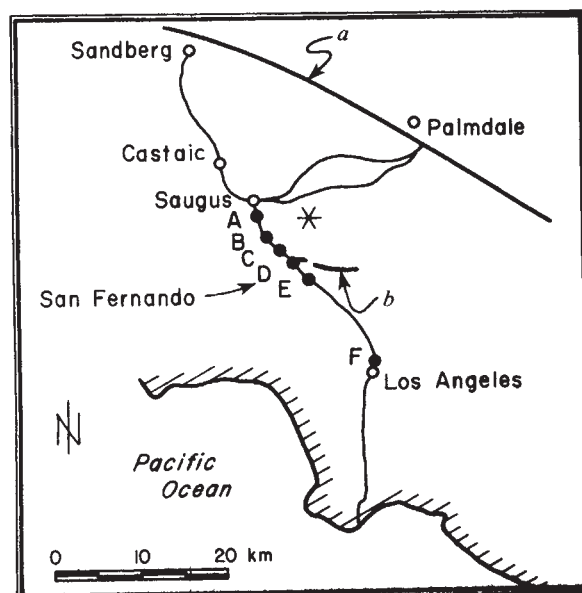
Figure 1 shows the location of the two levelling lines discussed by Castle *et al.*¹: Los Angeles–Saugus–Palmdale, and Los Angeles–Saugus–Sandberg. Figure 2 shows the uplift as a function of time at the points A–F (Fig. 1), which straddle the 1971 San Fernando thrust fault (Fig. 1). The similarity of the uplift history before the San Fernando earthquake (Fig. 2) and the famous uplift before the Niigata earthquake⁵ is striking. A 35–40-yr period of stability is followed by sudden uplift, which again is followed by a few years of quiescence. It is important to note that points A, B and C are located north of the San Fernando Fault on the upthrust block, and points D, E and F south of it on the downthrust block. All of these points were uplifted before the San Fernando earthquake.

This feature is required by the dilatancy hypothesis, and it is opposite to what is expected by the creep hypothesis, as will be discussed below.

In Fig. 2 it is also evident that the uplift started earlier in the north (points A, B), and was larger there, than in the south (points E, F). This sequence is examined in more detail in Fig. 3, which shows the uplift along the Palmdale–Saugus–Los Angeles traverse. The picture is that of an uplift disturbance initiated in the north (at the San Andreas) and spreading south with the San Fernando earthquake as a preliminary culmination. A tiltmeter located anywhere along this traverse would have recorded a south tilt at first (1961–64), and then a north tilt (1964–65), as was noted by Thatcher⁴ for the traverse Sandberg–Saugus.

The Sandberg–Saugus–Los Angeles traverse is shown in Fig. 4. For this traverse no data were available for the period 1961–64. During 1964–65, the uplift was concentrated in the middle to northern part of the traverse as it was for the other levelling line in the same period of time (Fig. 3). Next, the southern and northern portions were uplifted between 1965 and 1968. The middle portion stayed at the same elevation during this interval, but it caught up the next year, 1968–69 (Fig. 4c). The entire

Fig. 1 Map showing the location of levelling lines with respect to the San Andreas (a) and San Fernando (b) faults. *, 1971 epicentre. Uplift is measured with respect to the coastal point of the traverse (tidal 8) as constant¹.



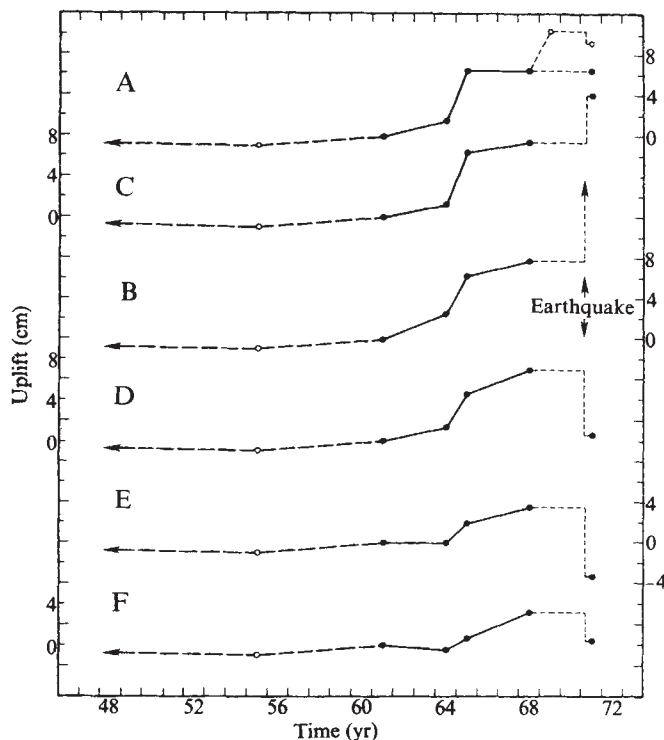


Fig. 2 Uplift as a function of time for three points each on the upthrust (A, B, C) and downthrust (D, E, F) blocks of the San Fernando quake. The data from 1961–71 are from Castle *et al.*¹. Before that, two measurements in 1926 (→) and 1955 (○) were the same, within a couple of cm, as those in 1961 (R. O. Castle, personal communication).

southern part of the traverse may also have been uplifted a further substantial amount in 1968–69; however, there are no data along this portion of the surveying line. The possibility of this additional surge in 1968–69 is indicated in Fig. 2 for point A as alternative. Figure 4d shows the elevations along the traverse after the 1971 San Fernando earthquake.

Interpretation

From Figs 2–4 it is evident that uplift before the San Fernando earthquake occurred both north and south of its surface rupture, suggesting that dilatancy preceded this earthquake. Dilatancy is the volume increase exhibited by sands and rocks under large deviatoric stresses^{6,7}, is measured as the deviation of the volume change, $\Delta V/V$, from the linear elastic behaviour, and is due to increased pore and crack volume. In the laboratory, dilatancy precedes rock failure. It was proposed that dilatancy in the crust is the cause of observed changes of crustal properties before some shallow earthquakes^{8–13}.

If we assume that the entire uplift was due to dilatancy, we may calculate the volume change from¹⁴

$$\Delta V/V \approx h/r$$

where h is the uplift and r is half the depth to which dilatancy extended. I will assume that only the 'brittle' part of the crust (the top 15 km which contain earthquakes) can become dilatant¹⁵. Under this assumption, a 15-cm uplift between 1961 and 1964 near the San Andreas implies that $\Delta V/V = 2 \times 10^{-5}$, which is schematically indicated in Figs 3a and 4a by dark shading. The spreading of increased uplift away from the San Andreas towards San Fernando is interpreted as being due to an increased amount of dilatancy, spreading through the crust, away from the San Andreas (see crustal cross sections as a function of time in Figs 3 and 4). Finally, the San Fernando earthquake locally released the deviatoric stress, and dilatancy disappeared below the southern portion of the profile. The

crust within 30 km of the San Andreas remained dilatant, in this interpretation, and the repetition of the 1857 earthquake will reduce this portion of the crust back to normal. This model also accounts for the tilt reversal, which was presented as one of the main supporting facts for the creep hypothesis⁴, and is in agreement with laboratory observations¹⁶, where localised bulges due to dilatancy are observed to migrate to different parts of the rock sample, and, in the final phase, begin to concentrate along the subsequent fracture plane.

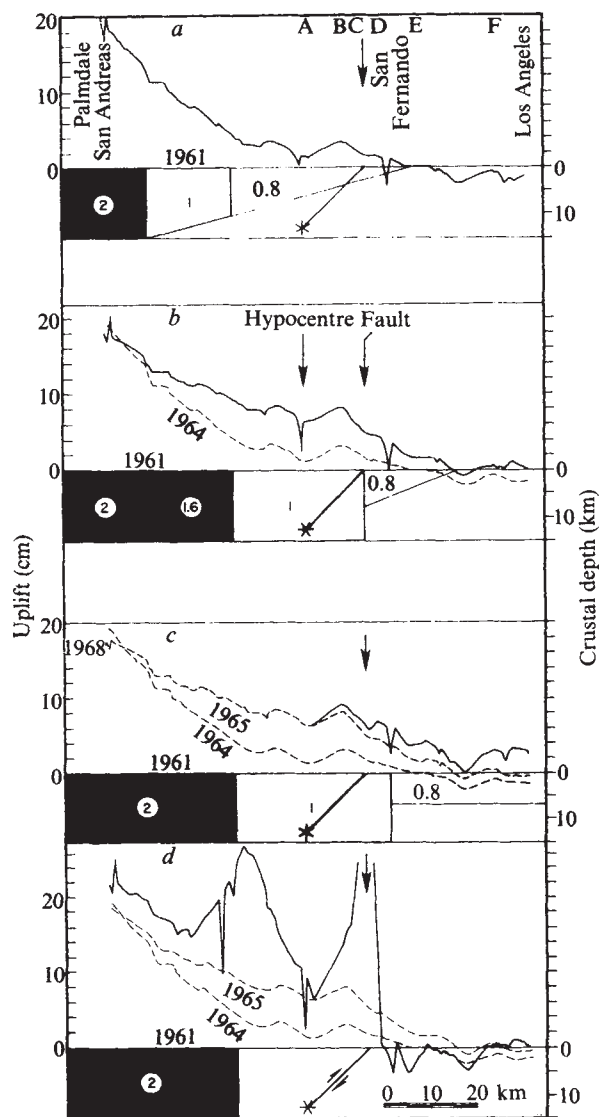


Fig. 3 Uplift along the Palmdale-Saugus-Los Angeles traverse from Castle *et al.*¹. The 1961 level was taken as reference with respect to which the 1964 (a), 1965 (b), 1968 (c) and 1971 (d) levels are shown. The state of the crust below the traverse is shown in each time frame. The number in the crust indicates the volume increase in units of 10^{-5} if it is assumed that thickness of the dilated crust is that indicated by the stippled area. In the 1971 curve the hump north of the hypocentre is due to the corner in this profile which re-approaches the epicentre at this point. a, Uplift between 1961 and 1964, decreasing gradually from 20 cm in the north (at the San Andreas) to zero in the south (San Fernando). b, Uplift in 1965 (—) with respect to the 1961 level. The 1964 uplift is retained (---) for comparison. No further uplift occurred between 1964 and 1965 in the north, but a substantial amount was added in the south, near the future San Fernando rupture. c, Uplift in 1968, again being concentrated even further south than the 1964–65 episode. Finally, after the 1971 San Fernando earthquake (d), the previously uplifted area south of the rupture was now below the initial level, north of the rupture large co-seismic uplift occurred, and along the San Andreas the uplift was essentially unchanged.

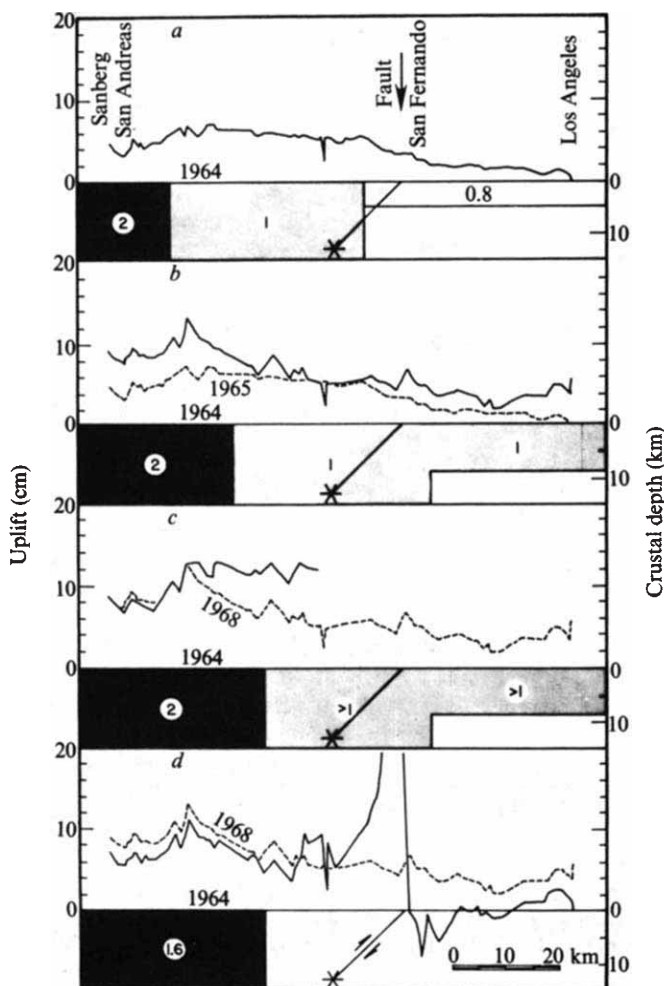


Fig. 4 Uplift along the Sanberg-Saugus-Los Angeles traverse from Castle *et al.*¹. The reference level is that of 1964. Levels are shown for: a, 1965; b, 1968; c, 1969; d, 1971. Uplift episodes are again approximately interpreted by an increasingly dilated crust. The amount of volume increase is again given in units of 10^{-5} with the assumption that in the time interval 1961-64, for which no data were available, an uplift of 10 cm occurred near the San Andreas, decreasing to zero at San Fernando. The basis for this assumption was the uplift between 1961-64 on the other profile (Fig. 3), but divided by two so as to be on the safe side.

Why should dilatancy begin at the San Andreas, and why should it persist there at a time when rupture occurred in a less dilated volume? Geodetic measurements in California suggest that strain accumulation may be concentrated along the San Andreas Fault^{17,22}. I will therefore assume that stresses are concentrated along the fault and hypothesise that the deviatoric stress C' , at which dilatancy appears⁷, was first reached near the San Andreas. As further deviatoric stress was accumulating, C' was exceeded in a widening crustal volume. Also, as Thatcher⁴ showed, the stress normal to the local trend of the San Andreas was increasing, thus locking the fault. The shear stress along thrust faults in the area increased, and so it follows that local thrust faults like the San Fernando Fault may fail first.

I also note that Thatcher⁴ has shown that the surface stress was everywhere compressive perpendicular to the uplift contours. This observation is in agreement with the laboratory measurements on dilating samples. Nearly always the stresses on the outside of the buckling samples are compressive, that is, the samples continue to become shortened throughout the experiments up to failure, even though dilatancy occurs.

Another interesting point is that there were two surges of uplift, one in 1961-65 and the other in 1968-69. The latter may have occurred only in the middle part of the profile, including the epicentre region of the San Fernando earthquake. The second phase of uplift could have been due either to the pore fluid pressure increase expected as a corollary of the wet dilatancy hypothesis¹⁸, or to precursory creep, as required by the dry dilatancy models.

Finally, the speed with which the uplift occurred might be taken to support the creep hypothesis. Laboratory results on jointed rocks¹⁹ suggest, however, that in crustal conditions, dilatancy uplift of a few centimetres could occur within a few months. Several years before earthquakes in Peru, Japan and California, approximately 6 cm of aseismic uplift occurred which accumulated within less than half a year²⁰.

Conclusions

The Southern California uplift can be explained by a dilatancy model, as well as by a creep model. In fact, aseismic uplift, the only direct evidence for dilatancy, can always be explained alternatively by a creep model, unless gravity observations are available together with uplift data.

Thatcher's⁴ creep model gives major importance to the transverse range. Its advantage is that it explains the observed asymmetry of the Southern California uplift (steeper to the south). Its disadvantages are that it does not explain the uplift south of the San Fernando rupture. The continuation of Thatcher's Fig. 4b to the south would have to show curves 1 and 2 below zero level, when the data show that this area was subject to uplift, not subsidence. Also, it does not explain why the uplift is centred on the San Andreas Fault, and it does not explain the Niigata data in a straightforward way.

The dilatancy model gives major importance to the San Andreas Fault as a plate boundary concentrating stresses. The transverse range follows from the bend in the San Andreas. This model's advantages are that it explains the entire uplift, including the uplift south of San Fernando. Also, it agrees with the interpretations of anomalous travel times¹³ and anomalous seismicity²⁴ before the San Fernando earthquake. The disadvantage of this hypothesis is that it does not explain the asymmetry of the uplift. It is quite plausible, however, that the rheological contrast between the granitic transverse range and the deep sedimentary basin of Los Angeles may cause a different dilatancy response to the same overall stress.

A test for accepting or rejecting one of the models could be found in the seismic wave velocities during the early and mid 1960s. The dilatancy model requires that in the early stage a velocity decrease should have occurred. From 1973 on, no substantial velocity anomalies are observed in the general area of the uplift²¹. If ground water has returned to fill cracks and pores, however, the anomaly would have disappeared by now. Another test lies in the future developments in the area. The creep hypothesis was developed on the example of a very small feature, the 20 km long San Fernando rupture. If the creep hypothesis is correct, it should apply to the entire length of the uplift, which is at least 200 km long. The corollary is that ten more San Fernando-type thrusts should occur in the future along the transverse range front. If the dilatancy hypothesis is correct, medium-sized thrusts are not excluded, but the San Andreas would be expected to produce a major quake with some subsidence following it.

I feel that neither the creep nor the dilatancy hypothesis is strong enough to be accepted at this point. While the uplift south of San Fernando is best explained by dilatancy, it may be quite likely that premonitory creep occurred on a deeper part of the San Fernando fault, as required by the dry dilatancy models.

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Hydrothermal fluids of seawater salinity in ophiolitic sulphide ore deposits in Cyprus

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Freezing points of fluid inclusions in vein quartz intergrown with sulphides from ophiolitic cupriferous pyrite ore deposits in Cyprus indicate a hydrothermal fluid of seawater salinity, and confirm the hypothesis of metal transport in hot seawater. It is therefore suggested that the economic potential of oceanic crust layer 2 is probably negligible.

EXISTING information suggests that hot seawater leached, transported and deposited metals (Fe, Cu, Zn, Pb, Ni, Co, Ag and Au) from solution to form the sulphide ore deposits in the Upper Cretaceous ophiolitic rocks of the Troodos Complex, Cyprus^{1–4}. The evidence is based principally on the use of isotopes (⁸⁷Sr/⁸⁶Sr, D/H, ¹⁸O/¹⁶O and ³⁴S/³²S) as geochemical tracers and is consistent with a model for hydrothermal metamorphism and mineralisation which involved convection of seawater within the ~2-km thick permeable layer of pillow lavas and dykes which forms the upper part of the ophiolitic sequence (Fig. 1)^{1–5}. By estimation of a Rayleigh number Spooner³ has shown that the fluid dynamic conditions necessary for vigorous hydrothermal convection were satisfied.

The ore deposits, which formed in the submarine environment, generally consist of a lens of massive sulphides (pyrite–chalcopyrite ± sphalerite ± marcasite ± galena) intercalated within the pillow lava sequence^{6–10}. This is underlain by a pipe-shaped mineralised stockwork extending downwards into the dyke complex (Fig. 1)^{6–10}. They have been worked for copper since pre-classical times, for high quality pyrite used in the manufacture of sulphuric acid¹¹ and for minor quantities of gold and silver, which occur in 'Devil's Mud' only found in the zone of supergene alteration⁶. Approximately 20 deposits containing on average ~1 million tonnes ore of variable copper content have been worked on the island. The largest and richest was Mavrovouni, which contained ~15 million tonnes ore grading between 3.5 and 4.5 weight % Cu (ref. 6). Limni—the largest working mine at present—produces ~450,000 tonnes ore per annum at a mean grade of ~0.85% Cu (refs 12,13).

The object of this article is to describe a test of the hypothesis of metal transport in hot seawater. This has involved determination of depressions of the freezing point of samples of the ore fluid which occur as small inclusions in minerals from the sulphide deposits. This colligative property, a reflection of the salinity of the solution, is then compared with the freezing point of seawater.

Experimental technique

The fluid inclusions were examined in thin sections of rock a few millimetres in size which were polished on both sides down to a thickness of ~60 µm using 1–0.3-µm sized alumina particles on a vibratory polishing machine. The slivers were prepared from ~1-mm thick slices of rock containing minerals with fluid inclusions which were mounted on glass slides using Lakeside resin. The thin sections were floated off by soaking in absolute alcohol for 1–3 days, and pre-selected areas of inclusions were cut out. Microthermometric measurements were then conducted on a Chaix–Meca heating–freezing (–180 °C to +600 °C) stage¹⁴ mounted on a Leitz Ortholux II microscope fitted with ×32 and ×50 UMK objective lenses and ×25 binocular eyepieces. This facilitated examination of inclusions down to a few micrometres in size. Measurements were carried out on about 20 inclusions per sample. The inclusions were pre-selected in order to obtain an approximately random sample.

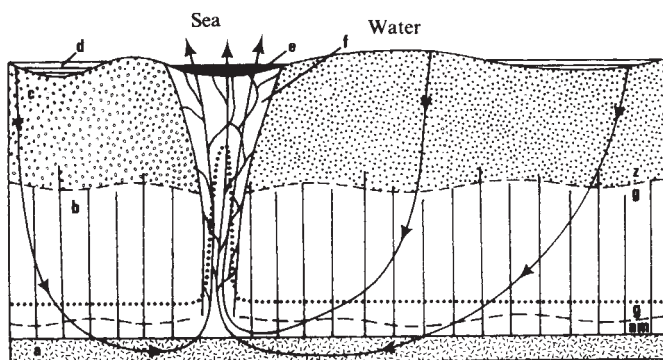
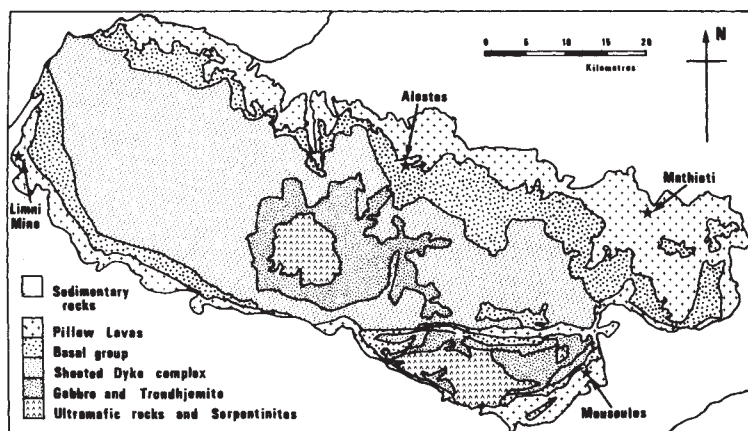


Fig. 1 Schematic diagram of model of hydrothermal convection, metamorphism and mass transfer within the ophiolitic rocks of the Troodos Massif, Cyprus. General metamorphism³⁷ occurred in zones of recharge flow, whereas formation of the cupriferous pyrite ore deposits^{6–10} occurred in the discharge zones. Precipitation of Fe/Mn enriched sediments ('umbers')^{38–40} was also probably a consequence of discharge of hot metalliferous solutions^{1,38}. a, Gabbros, metagabbro and variably altered trondhjemite; b, sheeted dyke complex; c, pillow lavas; d, umbers; e, massive sulphide ore; f, mineralised stockwork. Dashed lines approximate to metamorphic boundaries between zeolite facies (z), greenschist facies (g) and amphibolite facies (am). Continuous lines are schematic flow lines for hydrothermal convection; dotted line is approximate form of an isotherm produced by convection.

Fig. 2 Simplified geological map of the Troodos Massif, Cyprus (modified after the Cyprus Geological Survey map) showing the locations of the mines from which samples were obtained for fluid inclusion and strontium isotopic studies²¹.



Pure nitrogen gas, which was passed through a heat exchanger in liquid nitrogen, was used to control cooling. The sample was taken down to -50°C to ensure freezing of the inclusions since the fluid in the inclusions shows pronounced supercooling effects. Most inclusions froze at $\sim -30^{\circ}\text{C}$. The sample was allowed to warm up to a temperature $1\text{--}3^{\circ}\text{C}$ below the freezing point of the inclusions and was then usually refrozen to -21°C . This promoted growth of observable single crystals of ice and often produced a displacement of the vapour bubble from its equilibrium position. The temperature of ice disappearance was then measured in large inclusions ($\sim 50\text{--}100\text{ }\mu\text{m}$). In smaller inclusions ice was not in general visible¹⁵ because of the small refractive index difference between ice and water in dilute solutions. The temperature at which the vapour bubble returned to its original shape and position was then recorded. The freezing points of the inclusions were close to 0°C , so the apparatus could be precisely calibrated against the freezing point of deionised water. This was done by squirting a small quantity of vapour from deionised water over a sample at low temperature, to produce small crystals of ice immediately above fluid inclusions. The freezing points of the inclusions and the frost on the sample surface were then measured sequentially. Hence, the freezing points of the fluid inclusions and the deionised water were determined in identical experimental conditions. The result of 12 such measurements indicated that the recorded fluid inclusion freezing points had to be increased by 0.2°C . The estimated reproducibility of any given fluid inclusion freezing point is $\pm 0.2^{\circ}\text{C}$ for the particular temperature range encountered.

When fluid inclusions which were formed at temperatures greater than $\sim 50^{\circ}\text{C}$ cool down naturally vapour bubbles nucleate and grow, because the coefficient of thermal contraction of the liquid is greater than that of the enclosing solid. The temperature at which the vapour bubble disappears during controlled heating may then be determined in order to obtain an homogenisation temperature, which, if pressure corrected when necessary^{16,17}, gives a temperature of formation of the fluid inclusion. Such homogenisation temperatures are readily measured since the small vapour bubble goes into a state of progressively more violent Brownian motion just before its disappearance. The apparatus was calibrated using substances of known melting point^{14,15}. The accuracy of any given measurement was controlled by the observed range of temperature over which the standards melted, and is considered to be $\pm 2^{\circ}\text{C}$ for the temperature range encountered.

Fluid inclusion freezing points

As the primary salinity of the hydrothermal hot water was the subject of interest, it was necessary to avoid samples which could have contained inclusions formed from a

boiling fluid. Hence, material was examined from three relatively deep stockworks, in which pressure would have been highest. At Limni mine (Fig. 2) the main ore deposit occurs as a stockwork developed in pillow lavas^{15,18}. Samples were examined from the core of the deposit, where copper grades of $1\text{--}3\%$ occur. In the SE corner of the opencast pit pyritised and hydrothermally metamorphosed Sheeted Dyke Complex occurs as part of an unfaulted block. This locality is particularly valuable because it probably constitutes the best exposure of the deepest part of a stockwork anywhere in Cyprus. The metamorphic mineral paragenesis differs from that typical of higher level stockworks (quartz-chlorite-illite-sulphides¹⁰) and is characteristic of the greenschist facies (quartz-albite-chlorite-epidote-actinolite-sphene-pyrite). The third locality chosen was Alectos (Fig. 2) which consists of a small cupriferous pyrite stockwork developed in the zone transitional between sheeted dykes and pillow lavas (the 'Basal Group'). Three samples from each of these localities were studied.

Fluid inclusions were examined in vein quartz intimately intergrown with sulphides, which contains high densities of inclusions. The inclusions are of variable shape, some being very irregular in outline whereas others show well defined negative crystal morphologies. They are in general between 5 and $100\text{ }\mu\text{m}$ in size (Fig. 3a). Boiling during ore formation is usually indicated by the presence of two co-existing types of inclusion—one type, with a high vapour-liquid ratio, corresponding to 'steam' and the other, with a lower vapour-liquid ratio, corresponding to 'liquid'. The former homogenise in the gas phase, the latter in the liquid phase. Such evidence for boiling was searched for in these samples and not found. Most inclusions are of the simple two phase type, but a third solid phase was quite frequently present in larger inclusions. These daughter crystals occur as highly birefringent feathery aggregates similar in appearance to dawsonite ($\text{NaAl}(\text{CO}_3)(\text{OH})_2$)¹⁹ (Fig. 3a). The high birefringence suggests that they are some type of carbonate or sulphate.

The freezing points and homogenisation temperatures, most of which have been determined on the same inclusions, are given in Table 1 and all the freezing point data are presented as a frequency polygon in Fig. 4.

The inclusions are clearly of hydrothermal origin since their homogenisation temperatures are universally high. The data for the nine samples vary from $301 \pm 23^{\circ}\text{C}$ (21 measurements) to $351 \pm 4^{\circ}\text{C}$ (20 measurements). Most inclusions show no obvious geometrical relationships which can be used to indicate that they are primary (formed during growth of a crystal) or secondary (formed after growth). It could be argued, therefore, that they might be unrepresentative of the fluid which actually precipitated the ore minerals. This is shown not to be the case from examination of sample CY/75/39 from the main Limni

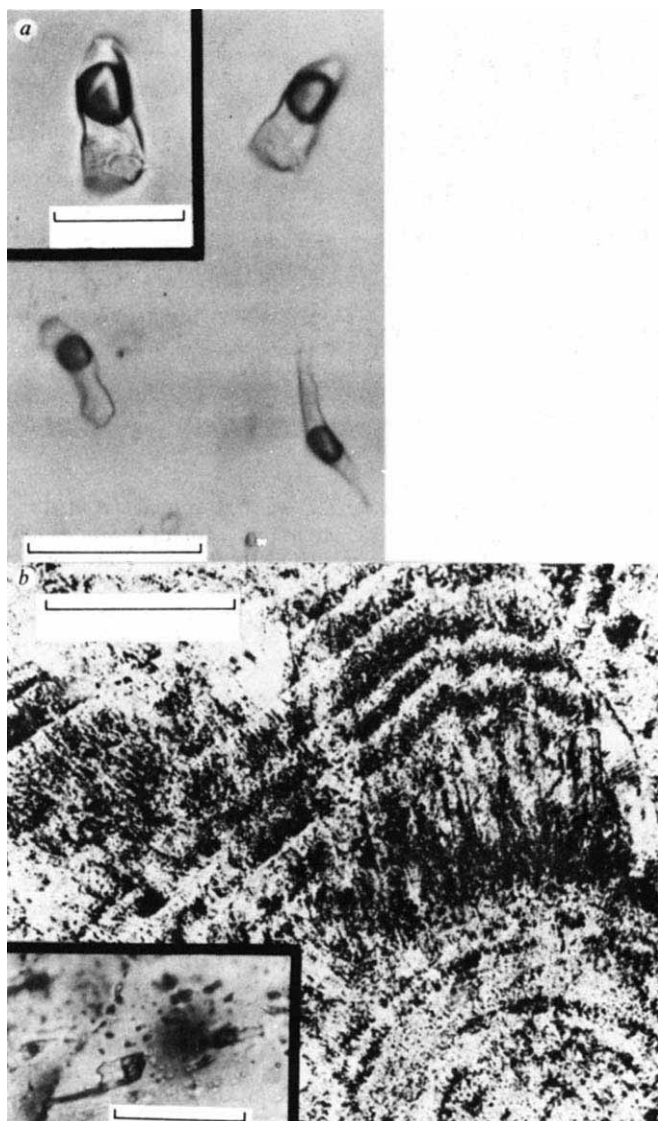


Fig. 3 *a*, Typical fluid inclusions in quartz from Cyprus sulphide ore deposit material (sample CY/75/38; Limni main stockwork; scale bar 30 μ m). Central upper inclusion; $T_H = 360^\circ\text{C}$, $T_F = -2.7^\circ\text{C}$. Lower left inclusion; $T_H = 313^\circ\text{C}$, $T_F = 2.1^\circ\text{C}$. Lower right inclusion; $T_H = 361^\circ\text{C}$, $T_F = -2.1^\circ\text{C}$. Inset shows daughter crystals (? carbonate or sulphate) in the central upper inclusion, scale bar 20 μ m. *b*, Primary fluid inclusions in vein quartz from the Limni main stockwork (sample CY/75/39). The inclusions define growth zones in the quartz crystal and are elongate parallel to the direction of growth. Therefore, according to normal geometrical criteria^{16,17}, they are considered to have formed during crystal precipitation. Since the vein quartz is intimately intergrown with sulphides the fluids in these inclusions are samples of the sulphide ore forming solution; scale bar 200 μ m. Inset shows two primary inclusions in detail; scale bar 35 μ m. Left inclusion; $T_H = 307^\circ\text{C}$, $T_F = -2.0^\circ\text{C}$. Right inclusion; $T_H = 309^\circ\text{C}$, $T_F = -2.0^\circ\text{C}$.

stockwork. This was the only sample to contain well defined primary inclusions clearly related to growth zones in quartz (see Fig. 3*b*). The results of measurements on these primaries and on inclusions of the more general type agree within 1σ (Table 1). Since the vein quartz is intimately intergrown with sulphides the fluid inclusion data from this sample, and all other samples, apply to the process of sulphide ore deposition itself.

The depressions of the freezing point of the fluid inclusions relative to pure water were found to be small ($-1.6 \pm 0.2^\circ\text{C}$ to $-2.4 \pm 0.4^\circ\text{C}$), and therefore indicate that the hydrothermal ore forming fluid was a relatively dilute solution (2.7–4.0 equivalent weight % NaCl). The data

for different samples are all statistically indistinguishable within 2σ limits, and most are indistinguishable within 1σ limits. Taking into account an accuracy of $\pm 0.2^\circ\text{C}$ for any given measurement, the frequency polygon for all the 205 determinations (Fig. 4) shows a unimodal and symmetrical distribution about a mean value of -1.9°C . The standard deviation is $\pm 0.4^\circ\text{C}$.

This freezing point is statistically indistinguishable from that of sea water. The salinity of sea water in the open ocean varies from 32 to 37.5‰ (ref. 20). In small ocean basins such as the Mediterranean and the Red Sea it rises to 41‰ (ref. 20). This produces a freezing point range for seawater of from -1.7°C to -2.2°C . Average seawater with a salinity of 35‰ has a freezing point of -1.9°C . Hence, it is clear that a hydrothermal fluid identical to seawater in its salinity formed the ophiolitic sulphide ore deposits of Cyprus. That this fluid was actually of seawater origin is indicated by the stable isotope work of Heaton and Sheppard⁴ and confirmed by the strontium isotope ratios reported by Chapman and Spooner²¹, which demonstrate ^{87}Sr enrichment of mineralised material up to Upper Cretaceous seawater $^{87}\text{Sr}/^{86}\text{Sr}$ values. The hypothesis of metal leaching and transport by hot seawater is, therefore, verified.

The data may be compared with freezing points of fluid inclusions in minerals from greenschist facies metabasic rocks dredged from the Atlantic Ocean floor¹⁵. Since the mean homogenisation temperatures of six samples containing secondary actinolite $\pm$ epidote range from 219°C to 324°C , the fluids in the inclusions are clearly hydrothermal in origin. The mean of 77 freezing point values given by Jehl¹⁵ for these samples is -2.0°C , and the

Fig. 4 Frequency polygon of the 205 freezing point measurements of fluid inclusions in vein quartz from ophiolitic sulphide ore deposits, Cyprus, compared with the freezing points of seawater²⁰ and of fluid inclusions in metabasic rocks containing actinolite $\pm$ epidote dredged from the Atlantic Ocean floor¹⁵. Mean freezing point -1.9°C , s.d. $\pm 0.4^\circ\text{C}$.

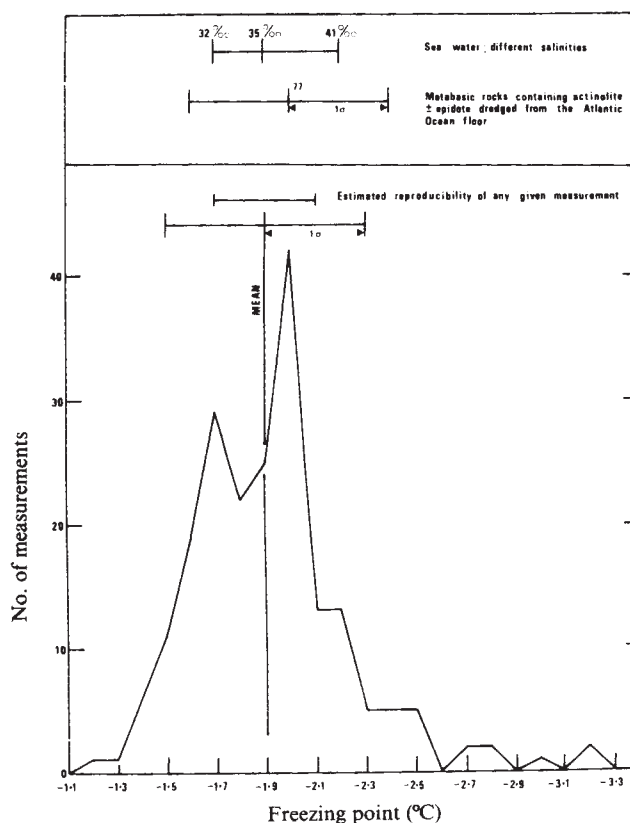


Table 1 Freezing points and homogenisation temperatures of fluid inclusions in quartz from the Limni and Alestos ophiolitic sulphide ore deposits and comparative data

Sample No.	Sample locality and description	Freezing point (°C)	Homogenisation temperature (°C)
CY/75/38	Limni, main deposit. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	-2.4 ± 0.4 (20)*	309 ± 26 (23)*
CY/75/39	Limni, main deposit. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	-1.7 ± 0.2 (20)	301 ± 14 (30)
CY/75/39 (Primary inclusions)	Limni, main deposit. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	-1.9 ± 0.2 (21)	307 ± 7 (21)
CY/75/52	Limni, main deposit. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	-1.6 ± 0.2 (22)	301 ± 23 (21)
CY/75/67A	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz-epidote-chlorite-actinolite-calcite-pyrite vein	-2.1 ± 0.4 (22)	309 ± 19 (21)
CY/75/67B	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz-epidote-chlorite-actinolite-calcite-pyrite vein	-2.0 ± 0.2 (19)	309 ± 22 (35)
CY/75/67C	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz-epidote-chlorite-actinolite-calcite-pyrite vein	-2.0 ± 0.2 (20)	322 ± 10 (24)
CY/75/98A	Alestos. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	-2.1 ± 0.5 (21)	351 ± 4 (20)
CY/75/98C	Alestos. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	-1.9 ± 0.3 (20)	314 ± 7 (21)
CY/75/98D	Alestos. Quartz-pyrite-chalcopyrite-haematite-chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	-1.6 ± 0.2 (20)	325 ± 26 (20)
Mean value of all freezing points		-1.9 ± 0.4 (205)	
Comparative data	Metabasic rocks containing actinolite $\pm$ epidote dredged from the ocean floor ¹⁵	-2.0 ± 0.4 (77)†	219–324†
	Kuroko sulphide ore deposits of Japan ²³	-1.8 to -3.7 ‡	146–189‡
	Sea water ²⁰	-1.7 to -2.2 §	
	Average sea water (salinity = 35‰)	-1.9	

*Mean $\pm$ s.d.; figures in parentheses are numbers of measurements.

†These data were obtained from six samples (GA 12-14, V27-DR, 11-K1, G12-19, D1-11, G12-A and AT150-20-1) listed by Jehl¹⁵. The number of measurements refers to the number of values quoted for each sample. The temperature range refers to the range of the means of the homogenisation temperatures listed for each sample. A wider range of salinities was recorded, but from material not from the greenschist facies^{15,41}.

‡This freezing point range was obtained from about 560 measurements on 51 samples from the Yunosawa deposit, Furutobe mine²³. Fluid inclusions were studied in barite, sphalerite and fluorite. 34 samples were from siliceous ore and 17 from black ore. The homogenisation temperature range given refers to the range of the means of 85 determinations on seven samples (five from siliceous ore and two from black ore) also from the Yunosawa deposit²³.

§This range was obtained from the salinity variation in the open ocean (32–37.5‰) and from the salinities found in small ocean basins such as the Mediterranean (37–40‰) and the Red Sea (40–41‰)²⁰.

standard deviation is ± 0.4 °C. This value is also statistically indistinguishable from that of seawater. These samples would probably have come from the recharge parts of ocean-floor hydrothermal convection systems, whereas the samples from the Cyprus ore deposits were formed in discharge zones. The data show, therefore, that seawater does not detectably change bulk composition sufficiently to affect its freezing point during interaction with basic igneous rocks at elevated temperature.

The freezing points of the Cyprus ore deposit fluid inclusions may also be compared with data from the Kuroko polymetallic sulphide deposits of Japan. These are also of submarine origin but formed in association with rhyolite domes rather than basic igneous rocks. Stable isotope ratios (δD and $\delta^{18}O$) of fluids from inclusions in sulphides in these deposits suggest that the ore fluid was also predominantly of seawater origin²². The range of ~ 560 measurements of freezing points of fluid inclusions in barite, sphalerite and fluorite from the Yunosawa deposit, Furutobe mine has been reported²³ as -1.8 °C to -3.7 °C (34 samples were from siliceous ore and 17 from black ore). This is very similar to the total range of fluid inclusion freezing points from the Cyprus ore deposits (-1.2 °C to -3.2 °C; Fig. 4). Hence, the effects on the

bulk composition of seawater during water-rock interaction in the Cyprus and Kuroko environments were essentially the same.

Economic potential of oceanic crust layer 2

This information on fluid inclusion salinities in Cyprus ore deposits shows that when ordinary seawater interacts at elevated temperature with ordinary basaltic rock, sulphide deposits can be formed. Hence, regardless of whether the ophiolitic sequence of the Troodos Complex, Cyprus, is or is not a representative tectonically emplaced fragment of oceanic crust and upper mantle^{24–26}, the ore deposits within it may probably be taken as characteristic of the type of ore deposit formed during seawater circulation^{1, 27–30} within spreading oceanic ridges. This convective process is now thought to occur on a massive scale, and is probably the main hydrothermal ore forming process which affects the upper part of the sequence of igneous rocks of the oceanic crust (layer 2).

Hence, the sizes and metal contents of the Cyprus deposits may be used to assess the possible economic significance of oceanic crust layer 2. As mentioned above, these deposits are essentially small (0.5–15 million tonnes), occasionally relative rich copper ore bodies (~ 0.5 – $\sim 4\%$ Cu), which

contain no other metals of economic importance⁶⁻¹³. To place them in context, they may be compared with a class of hydrothermal ore deposit which supplies the largest contribution (~45%) to world copper production—the porphyry copper deposits³¹. The average size of porphyry coppers is approximately 150 million tonnes, and they normally contain greater than 0.5% copper (average ~0.8% Cu) which is often associated with economically significant quantities of molybdenum or gold³². They are therefore on average 1–2 orders of magnitude larger than Cyprus-type deposits, and can be much bigger. For example, the largest worked porphyry copper deposit (Bougainville, Territory of Papua and New Guinea) contains 900 million tonnes ore grading 0.48% Cu and 0.6 p.p.m. Au (ref. 33). The largest known porphyry copper (Cerro Colorado, Panama) contains at least 2,000 million tonnes ore grading 0.61% Cu (ref. 34). These data show, therefore, that Cyprus-type deposits are not particularly economically significant even when exposed on land. Also, an additional source of supply of copper in the future will probably be obtainable from the 'manganese nodules' which occur on the ocean floor^{35,36}.

Hence, bearing in mind the technological difficulties of ocean floor mining, this brief assessment of the ophiolitic sulphide ore deposits of Cyprus suggests that it is unlikely that layer 2 of the oceanic crust contains economically significant mineral deposits.

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Subsurface movements of stone artefacts and their implications for the prehistory of Central Africa

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The general and systematic character, in Central Africa, of a postsedimentary process of vertical redistribution of stone artefacts buried in a Kalahari sand mantle is strongly suggested. This invalidates the prehistoric nomenclature and chronology of this area and may have implications on the palaeoclimatological interpretation of stone-line profiles.

THE immense area occupied by the Zaïre or Congo river basin in the heart of Africa is still very little known from the archaeological point of view. The present-day nomenclature of the post-Acheulian prehistoric industries of Central Africa is based on very few excavations and on some systematic surface collections, essentially made in the regions of Kinshasa, in Zaïre¹ and of Lunda, in north-eastern Angola². This nomenclature presents a somewhat conjectural and theoretical character as it consists only of a compilation of various observations made at different sites. Only at the site of the Gombe Point (formerly Kalina Point)

at Kinshasa, which had been excavated³⁻⁴ as early as 1925, was a complete post-Acheulian succession supposed to exist.

In 1968, a Symposium held at Tervuren, Belgium, under the auspices of the Commission of Nomenclature and Terminology of the Pan-African Congress on Prehistory and Quaternary Studies expressed the wish that new excavations should be undertaken at Gombe Point to determine and date this prehistoric succession⁵. These new excavations⁶ were performed in 1973 and 1974. Fairly quickly, the conclusion was reached that this new research raised a series of problems much more important than the mere revision of the prehistoric nomenclature as it now seems that all the evidence, previously considered to be established, should be questioned.

Evidence from the Gombe excavations

At Gombe, the stone artefacts are distributed irregularly at all depths but with a considerable concentration (45%–95% of the total population) near the base of a homogeneous sand-silt-clay mantle, 3–5 m thick, which overlies a silicified bed-rock. This mantle is derived from the Red Sands of the Upper Kalahari, which cover the surrounding

Table 1 Correlation of the radiocarbon dates obtained at Gombe Point and in the regions of Kinshasa and Lunda

Industries	Gombe	Kinshasa	Lunda
-UPPER	GrN 7,219 : 3,385 ± 35 b.p. GrN 7,278 : 5,260 ± 40 b.p.	Lv 288 : 3,870 ± 90 b.p. Lv 162 : 5,750 ± 110 b.p. Lv 45 : 6,030 ± 190 b.p. Lv 289 : 6,280 ± 130 b.p. Lv 165 : 8,920 ± 160 b.p. Lv 164 : 9,730 ± 200 b.p.	UCLA 171 : 4,700 ± 100 b.p. UCLA 167 : 6,830 ± 120 b.p.
TSHITOLIAN			
-LOWER			UCLA 172 : 12,970 ± 250 b.p.
LUPEMBO/TSHITO- LIAN OR	GrN 7,220 : 8,095 ± 50 b.p.	Lv 287 : 12,230 ± 250 b.p.	C 580 : 11,189 ± 490 b.p.*
UPPER LUPEMBAN	GrN 7,276 : 14,840 ± 80 b.p.	Lv 166 : 15,080 ± 480 b.p.	C 581 : 14,503 ± 560 b.p.
SANGOAN/ LOWER	GrN 7,221 : 27,240 ± 280 b.p.	Lv 163 : > 26,000 b.p. Lv 44 : > 30,000 b.p.	UCLA 169 : > 34,000 b.p. UCLA 168 : 38,000 ± 2,500 b.p. UCLA 709 : > 40,000 b.p.
LUPEMBAN	GrN 7,277 : > 43,800 b.p.		

*This sample is associated with a material identified as Lower Tshitolian or Lupembo-Tshitolian.

highlands⁷. This kind of irregular distribution of artefacts and this type of sediment are typical for the great majority of the open air archaeological sites known in the southern part of the Zaïre river basin and on which the knowledge of the Prehistory of Central Africa relies⁸.

A study of the typological and quantitative distribution of the artefacts according to depth seemed to suggest the existence of different prehistoric industries: in the different trenches, various assemblages of characteristic artefacts were found in more or less the same order from the top to the base of the sand mantle. Charcoal fragments found at the same levels revealed dates which fell within the same periods as those already obtained for analogous industries from Kinshasa and Lunda. A fair typological and chronological correlation could thus be noted between the industries of the southern Zaïre river basin (Table 1). It must, however, be emphasised that at Gombe, just as at Kinshasa and Lunda, the dated samples exhibited no other relation with the archaeological material than to have been found at the same depth as some characteristic artefacts.

However, a systematic study of the reassembly of the worked stones of Gombe (that is, joining together the tools, flakes, core and fragments struck off from the same block) has shown that fitting artefacts were found at several different depths from which different radiocarbon dates have been obtained (the vertical distance between joining pieces sometimes exceeds 1 m). Hundreds of analogous observations have been made through the whole thickness of the sand mantle.

Fitting artefacts are part of the same technological process (blades removed from a blade core for instance) and are strictly contemporaneous. In most cases, the vertical distribution of the elements of the reconstructed cores does not follow the order in which they have been struck off

(Fig. 1). This excludes the hypothesis of a later reutilisation of older artefacts. The abundance of reassembled pieces also precludes important lateral transport. Finally we were led to the hypothesis that only subsurface movements of the worked stones could explain this important vertical dispersion of contemporaneous artefacts. On the evidence of the reassembly, it becomes clear that, contrary to appearances, the site of Gombe Point has been disturbed in some way and that the lithic assemblages discovered there at a given depth are not homogeneous. As the Gombe Point can be considered as one of the most complete and most representative prehistoric sites of the southern Zaïre basin, it was thought important to investigate more deeply these disturbances.

Experimental research

By means of experiments⁹, the possibility of subsurface movements¹⁰⁻¹¹ and of a dispersion of artefacts was tested out. The original materials were used for the experiments. The texture and some mechanical properties of the sediment are given in Table 2.

We investigated whether artefacts, laid down on and/or buried in the sediment could undergo vertical movements, when the sediment is wetted and dried alternatively. Down to depths of 60 cm sand columns were used. Greater depths were simulated by putting down weights on the sediment column. The different methods of wetting and drying were

Table 2 Characteristics of the sediment used in the experiments

Sand content (> 63 µm) ²	58.1%
Silt content (2-63 µm)	28.0%
Clay content (< 2 µm)	13.9%
Median size	77 µm
Modal size	95 µm
Calibrage	very bad
Liquid limit water content	23%
Plastic limit water content	12%
Plasticity index	11%
Specific weight	2.63 g cm ⁻³
Angle of internal friction	45°
Cohesion	0 g cm ⁻² } water content: < 1%
Apparent angle of internal friction	15°
Apparent cohesion	50 g cm ⁻² } water content: between 20% and 23%
Compressibility constant:	
dry	25.9
wet (19% water content)	25.6 } slow test
Permeability: 10 ⁻³ cm h ⁻¹ for a dry volume weight of 1.42 g cm ⁻³	

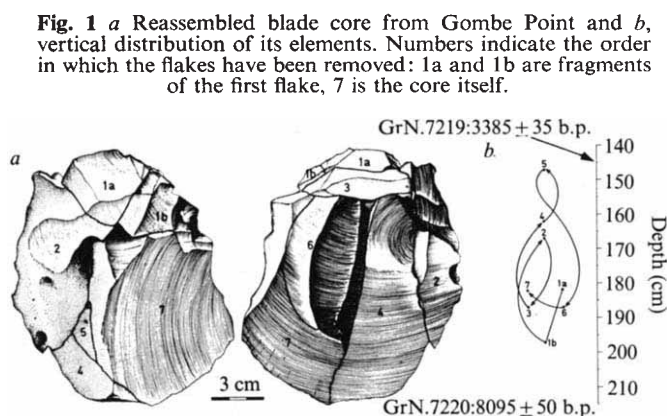


Fig. 1 a Reassembled blade core from Gombe Point and b, vertical distribution of its elements. Numbers indicate the order in which the flakes have been removed: 1a and 1b are fragments of the first flake, 7 is the core itself.

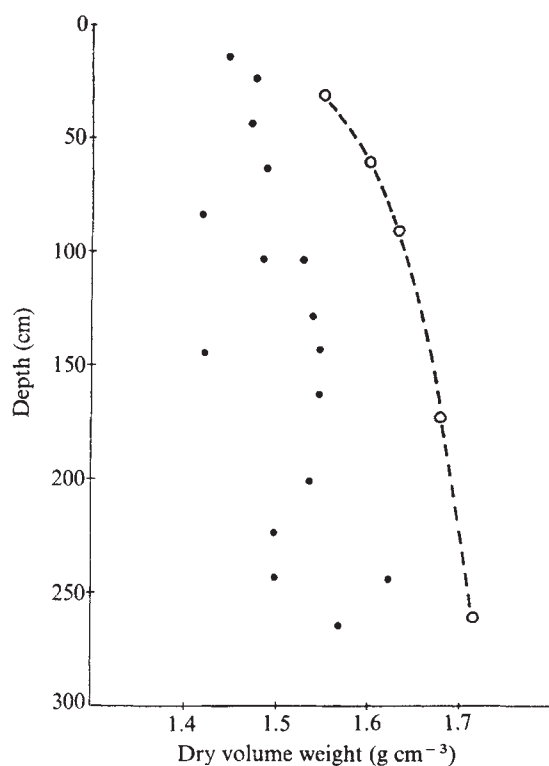


Fig. 2 The dry volume weight of undisturbed soil samples taken from trench 2 at Gombe (●) and the dry volume weight derived from the experiments and the oedometer-test (dotted line). In establishing the latter, a supposed dry volume weight of 1.5 g cm^{-3} of the column above each point was accepted. Therefore the dotted line still gives underestimated values.

simulations of an oscillating water table or of the percolation of rain water from the surface. Three conclusions were reached:

(1) Artefacts laid down on the surface can penetrate into the sediment over a distance of some mm to more than 1 cm, depending on their weight and shape. The amount of penetration shows a linear relation with the logarithm of the number of wetting-drying cycles applied. The explanation lies in a slight compression of the sediment just beneath the artefact. The experiment showed that the weight of the artefacts becomes unimportant and negligible, once they are covered by some cm of sediment.

(2) Buried at greater depths, artefacts do not move vertically, except if the artificial soil column is not entirely consolidated. Artefacts, laid down in an unconsolidated sediment at real or simulated depths of 30, 60, 90 and more than 200 cm, displayed, during consolidation, a general tendency to accompany the descent of the level at which they were buried. Some of them did however descend considerably more or less than the sediment which originally surrounded them.

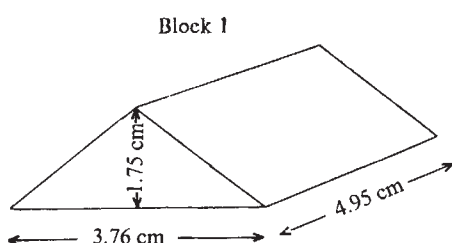
(3) The dry volume weight of the sediment, measured in the basal part of the different experimental boxes after each experiment, exceeds systematically that of undisturbed samples, taken in the field at depths obtained or simulated in the laboratory (Fig. 2). An oedometer test confirmed the dry volume weight values obtained from the experiments.

The observation in the field of termite activity and the fact that undisturbed soil samples displayed in several cases a high density of very small (ϕ about 0.5 mm) biogenic galleries led to the conclusion that the sediment mantle at Gombe is still unconsolidated. It undergoes a double post-sedimentary process: the soil fauna brings up particles to the surface. The dry volume weight of the sediment will diminish along collapsing biogenic structures. A continuous biogenic action and subsequent collapse of the structures

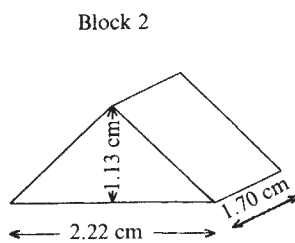
Table 3 Movement indices as measured in the shearing box of a shearing apparatus

Block number	Sediment	Orientation of block	Number of attempts	Mean movement index
1	wet	point up	6	1.16 ± 0.12
	wet	point down	10	0.89 ± 0.21
	dry	point up	5	1.51 ± 0.21
	dry	point down	5	1.28 ± 0.21
2	wet	point up	5	0.95 ± 0.14
	wet	point down	5	0.97 ± 0.15
	dry	point up	5	1.25 ± 0.16
	dry	point down	5	1.38 ± 0.17
3	wet	point up	5	1.18 ± 0.05
	wet	point down	5	0.98 ± 0.08
	dry	point up	5	1.37 ± 0.24
	dry	point down	5	1.28 ± 0.20

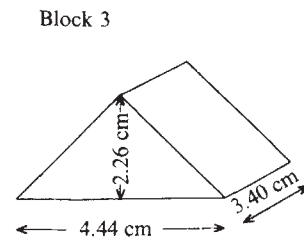
The movement index $i = (\Delta h_0)/(\Delta h_s)$, where Δh_0 is the absolute amount of descent of the gravity centre of the object during exertion of vertical stress on the soil column; Δh_s is the absolute decrease in height of the part of the soil column situated before the measurement between the bottom of the box and gravity centre of the object. For $i < 1$ the object penetrates in sediment situated originally above. In relative terms, the object rises. For $i > 1$ the object penetrates in sediments situated originally below. In relative terms, the object descends.



Centre of gravity 1.24 cm below top



Centre of gravity 0.80 cm below top



Centre of gravity 1.60 cm below top

created in this way, must finally lead to a reconsolidation of the soil column. In fact, the sediment mantle at Gombe must be considered as consolidating permanently in reaction to the continuous biogenic upworking.

In this perspective, conclusion (2) (see above) becomes very significant and was therefore elaborated on by further experiments, simulating the consolidation of the sands by applying vertical stresses. Measurements were made in the shearing box of a monoaxial shearing apparatus: it was noted on several occasions that undeformable objects did not, under compression, strictly accompany the vertical movement of the enclosing sediment. Depending on dimension, form and orientation of the objects and on the water content of the sediment, the objects penetrated into sediments originally situated above or below them. Table 3 gives the vertical movement indices for three wooden blocks.

It should be pointed out that the variability in shapes and dimensions of a lithic industry is obviously considerably higher than that of three wooden blocks. Therefore, the range of vertical movement indices within a whole industry will be also much wider than for the three blocks. Moreover, the experiments are based on the theoretical conception of a homogeneously spread biogenic activity. This is not the real situation in nature. Preferential reworking can contribute to a much higher dispersion than expected on the base of the experiments. Finally, these experiments yield information only about dispersion during consolidation. It is clear that the biogenic activity itself can also create vertical displacement of artefacts.

Discussion

At Gombe, the reassembly shows that strictly contemporaneous artefacts are scattered irregularly over the whole thickness of the homogeneous sand mantle. The experiments confirm the possibility of a postsedimentary differential descent of artefacts in the soil column. This process is catalysed by biogenic activity.

The following facts strongly suggest that this process of redistribution has a general and systematic character in the entire area covered by Kalahari-type sands in Central Africa:

- (1) The sites on which our present knowledge of the Prehistory of Central Africa relies are located in the same environmental background—the southern Zaïre river basin.
- (2) In these sites, the artefacts are generally distributed in a similar way—"isolated and at no consistent level"¹⁸—

within a homogeneous mantle of redistributed Kalahari sands.

- (3) At Lunda, Kinshasa and Gombe, typologically identical assemblages of worked stones are dated within exactly the same periods.

- (4) Although systematic reassembly of lithic material has only been carried out for Gombe, similar examples of joining pieces found at different depths have also been observed at Dinga (Kwango) and at Kamoa (Shaba)¹², thus proving that Gombe is not an isolated case.

The most evident result of this redistribution process is a mixing up of originally superposed occupation layers and in most cases the inability to decide if one or more industries are present. The post-Acheulian industries that have been defined in Central Africa probably represent nothing more than heterogeneous assemblages of stone artefacts, without any stratigraphical, typological, chronological or ethnographical significance. The radiocarbon dates give only the age of various charcoal and wood samples but have no direct relation with the archaeological material.

The redistribution process also presents an interest for Quaternary stratigraphical interpretations in the area considered here. Part of the basal concentration of artefacts at Gombe cannot be interpreted as a residual pavement. The contemporaneity of several pieces of this concentration and of some artefacts scattered in the overlying sand mantle can only be explained by a differential descending velocity: artefacts with higher movement indices have already got down to the bed-rock, while others are still descending. The distribution of coarse elements in many stone-line profiles in Central Africa resembles that of the artefacts at Gombe. Many of these stone-lines could perhaps be considered as the result of descent through the overlying mantle¹⁰⁻¹¹, instead of a residuum, concentrated during an erosion phase. This has implications for the palaeoclimatological interpretation.

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Defective interfering particles with covalently linked $[\pm]$ RNA induce interferon

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Defective interfering (DI) particles of vesicular stomatitis virus which contain covalently linked complementary $[+]$ message and $[-]$ anti-message RNA as a single-stranded ribonucleoprotein complex within the particle, are extremely efficient inducers of interferon. A single particle can induce a quantum yield of interferon. A single molecule of double-stranded RNA presumed to form, at least in part, on entry into the cell is thought to induce interferon synthesis. Conventional $[-]$ RNA DI particles with the same polypeptide composition as $[\pm]$ RNA DI particles fail to induce interferon.

LAZZARINI *et al.*¹ have described a defective interfering (DI) particle of vesicular stomatitis virus (VSV), DI-011, which contains covalently linked complementary $[+]$ message and $[-]$ anti-message (genomic) RNA. The $[\pm]$ RNA exists within the particle as a single strand of 8×10^5 daltons in a ribonucleoprotein complex 0.85 μ m long, and on deproteinisation 80-90% of the RNA self-anneals into a double-stranded (hairpin) structure 0.33 μ m long¹.

The availability of pure populations of these defective interfering particles which contain RNA capable of assuming a double-stranded configuration and which can specifically bind to and enter cells, provides a unique system for delivering a

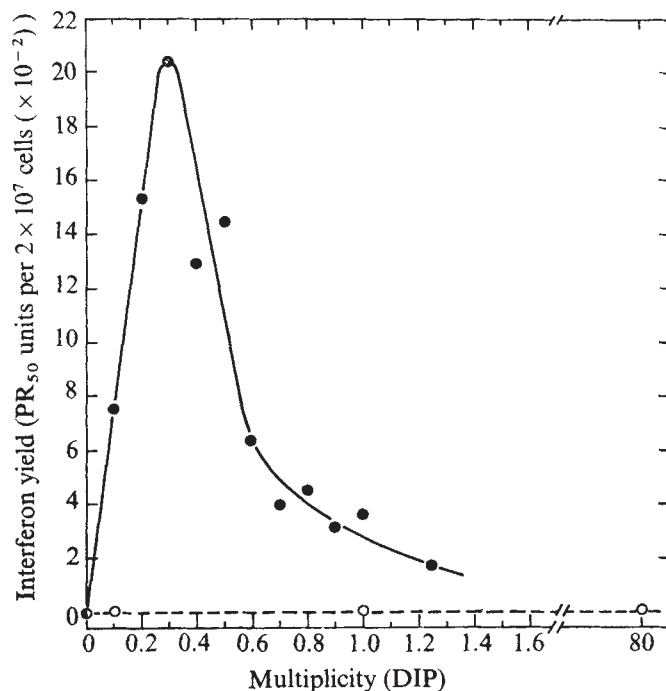


Fig. 1 Interferon inducing capacity of [$\pm$]RNA DI-011 (●) and [-]RNA DI-HR (○) particles. Primary chick embryo cells aged *in vitro* for 6 d (ref. 6) were infected with varying multiplicities of DI particles obtained as thrice gradient-purified stocks. DEAE-dextran at $10 \mu\text{g ml}^{-1}$ was present during virus attachment. Following virus attachment at 37.5°C for 30 min cell monolayers were washed three times with warm medium and incubated at 40.5°C with 3 ml of attachment solution²⁸ per 60-mm dish. After 24 h the supernatant fluid was collected and assayed for interferon as described previously¹⁵. Activity was recorded as a 50% VSV plaque reduction unit (PR_{50} unit ml^{-1})¹⁵. Interferon yield is reported as the total amount of interferon released into the medium from a monolayer of 2×10^7 cells in 24 h. This period sufficed to produce maximal yields of interferon over the entire range of multiplicities tested. DI particle titres were determined by measuring the reduction in yield of thrice gradient-purified homologous VSV-HR B particles^{8,9}.

single molecule of [$\pm$] stranded RNA to the cell in conditions which preclude RNA replication². We have used this system to determine whether the [$\pm$] RNA of DI-011 particles might assume in the cell a configuration of biological significance beyond that of its role as a DI particle. We examined the function of DI-011 as a defective cell-killing particle^{3,4} in conditions where conventional [-] strand RNA particles do not kill cells³, and tested their capacity to induce interferon production^{5,6}. We present evidence here that DI-011 particles do not kill cells, even at multiplicities of infection up to 50, but that a single particle, presumably because of its potential to form double-stranded (ds) RNA and the capacity of dsRNAs to induce interferon^{5,7}, suffices to induce a quantum yield of interferon, making it the most efficient inducer of interferon discovered so far.

Interferon induction by DI-011

Primary chick embryo cell monolayers aged *in vitro* for 6 d (ref. 6) were challenged with various multiplicities of DI-011 particles (m_{DIP}), on the basis of interfering particle activity^{8,9}. Our basic observation is illustrated in Fig. 1 as a dose (m_{DIP})-response (interferon yield) curve and demonstrates that preparations of DI-011 particles contain highly efficient interferon inducers. These data, typical of seven experiments, reveal that initially, increasing the multiplicity of DI-011 particles produced a sharp increase in the yield of interferon, with peak titres (at 24 h) obtained at about $m_{\text{DIP}} = 0.3$. (This value ranged from $m_{\text{DIP}} = 0.1$ to 1 in seven experiments.) Further

increases in m_{DIP} resulted in a marked reduction in interferon yield. The magnitude of the changes in interferon yield observed on increasing the dose of DI-011 particles in the low multiplicity range seemed characteristic of dose-response curves where one particle elicits a quantal response but two or more do not. Consequently, we compared experimentally derived dose-response curves with those expected from a Poissonian distribution of DI particles for cases where quantal yields of interferon would derive from classes (subpopulations) of cells attaching r particles at different multiplicities, m , and values of r (ref. 10). In Fig. 2 we compare the expected curve from the Poisson distribution for the class of cells infected by only one particle ($r = 1$) with the observed data from two experiments. The multiplicity of DI-011 particles which produced the maximum yield of interferon was determined by the least squares method for a best fit curve, with the logarithm of the interferon yield used to minimise the percentage error in the assays for high and low values of interferon. Curves representing other values of r ($r = 2$, $r > 1$, $r > 2$) are not shown, but revealed marked deviation from the observed results. From the goodness of fit of the observed dose-response curve to that expected from the class of cells infected with only one particle of DI-011 ($r = 1$), we conclude that infection with a single particle of DI-011 suffices to induce a quantum yield of interferon—in effect, defining an ‘interferon-inducing (IF) particle’. An equivalency between interferon-inducing particles and physical particles is implicit in Lazzarini’s observation that

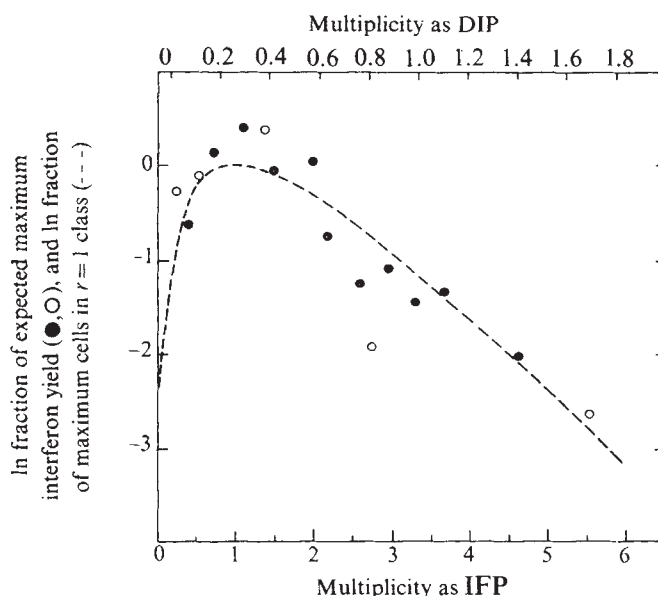


Fig. 2 Comparison of the observed dose (multiplicity)-response (interferon yield) data with those expected if only cells which received one particle were full yielders of interferon—representing the $r = 1$ class of the Poisson distribution where

$$P(r) = (e^{-m} m^r)/r!$$

In this case, m is the multiplicity of DI-011 particles, and $P(r)$ the fraction of cells infected by r particles of DI-011. The class of cells which is infected by a single particle (that is, the case where $r = 1$) is $P(1) = m e^{-m}$ (ref. 10). ●, ○, Data from Fig. 1 and one additional experiment. ---, Curve derived from the $r = 1$ class of the Poisson distribution. The upper abscissa shows the multiplicity of infection based on the DI particle titre whereas the lower abscissa defines the DI-011 stock in terms of the number of interferon-inducing (IF) particles. On the ordinate, the ‘expected’ maximum interferon yield was based on a maximum derived from a least squares fit of the experimental points. For these experiments the ratio IFP:DIP ≈ 4 , and indicates that more virions can function as interferon inducers than as DI particles. In this regard, Lazzarini consistently finds at least a threefold excess of physical particles, as assayed by electron microscopy, to DI particles, as measured by the interference test^{8,9} (personal communication). Allowing for this factor of three, the multiplicity on the top and bottom abscissae become almost the same, indicating an equivalency of physical and IF particles.

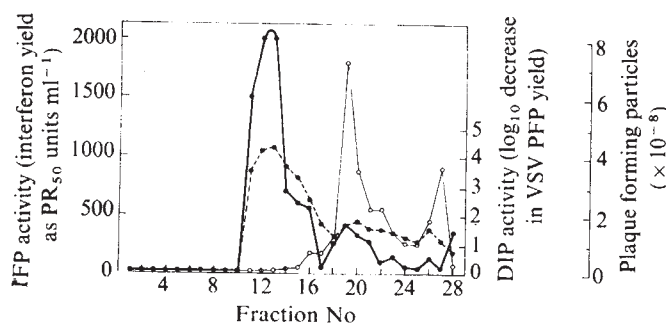


Fig. 3 VSV-MS(DI-011) profiles of plaque-forming particle (○—○), defective-interfering particle (●—●) and interferon-inducing particle (●—●) activity following velocity sedimentation on a linear sucrose gradient. A partially purified preparation of VSV-MS(DI-011) labelled with ^3H -uridine and containing roughly equivalent amounts of B and T particles (kindly supplied by R. A. Lazzarini) was applied to a 17 ml 5–40% linear sucrose gradient and centrifuged at 75,000g for 120 min at 4 °C. Fractions were collected from the top of the gradient and analysed for TCA-precipitable radioactivity (data not shown), plaque-forming particle, defective-interfering particle and interferon-inducing particle activity as described elsewhere³. Assays of interferon inducing activity for each fraction were carried out at optimal multiplicities for interferon induction (Fig. 1).

there is about a threefold excess of physical particles to defective-interfering particles in purified preparations of DI-011 (R. A. Lazzarini, personal communication). The data also indicate that cells which attach two or more DI-011 particles yield little or no interferon. Factors affecting the production (synthesis and release) of interferon and an antiviral state in this multiplicity range will be reported elsewhere.

Conventional [—] RNA particles representing a common one-third length component from the Indiana HR strain of VSV did not induce interferon when tested similarly (Fig. 1).

Comparison of different types of particle

A stock containing DI-011 and standard infectious B particles was obtained from BHK-21 cells in conditions which produced roughly equivalent amounts of DI and B particles. These particles were concentrated by centrifugation, and profiles of interferon-inducing, defective-interfering and plaque-forming particle activity established by testing each fraction of the gradient for these respective activities as described previously³ (Fig. 3). These results demonstrate that the interferon-inducing and defective-interfering particle activity profiles are essentially coincident throughout the gradient, with both activities peaking at fractions 12 and 13. Infectivity in the form of plaque-forming particles peaks at fraction 19. We conclude that the interferon-inducing particle activity of DI-011 particle preparations corresponds in sedimentation characteristics to those of defective-interfering particles.

Prevention of interferon induction

Inhibitors of cellular RNA and protein synthesis, such as actinomycin D and cycloheximide respectively, prevent interferon induction⁵. Table 1 demonstrates that when either of these drugs is present together with an optimal multiplicity of DI-011 interferon-inducing particles, little or no interferon is produced. Thus, it seems that new cellular mRNA and protein synthesis are required for the production of interferon which follows infection with DI-011 particles.

When preparations of DI-011 particles are exposed to anti-serum specific for the Indiana serotype of VSV, there is a marked reduction in the capacity of that preparation to induce interferon (Table 1). We conclude that the molecule(s) responsible for interferon induction in populations of DI-011 particles gain entry into the cell through particles which can be specifically neutralised with VSV antiserum.

Effects of heat or ultraviolet radiation

Stocks of gradient-purified DI-011 particles were exposed to different doses of ultraviolet or heated for 20 min at 50 °C and tested for their capacity to induce interferon. Table 1 shows that heated DI-011 particles remained fully functional as interferon inducers. The same heat treatment when applied to complete virions reduced infectivity, cell-killing particle activity, and virion-derived transcription 100-fold⁴. Figure 4 shows that the interferon-inducing capacity of DI-011 particles is extremely resistant to inactivation by ultraviolet. A dose of ultraviolet (2,615 erg mm⁻²) equivalent to 50 and 10 lethal hits to plaque-forming and cell-killing particle activity, respectively⁴, had virtually no effect on interferon induction by DI-011 particles, but reduced the interfering capacity of DI-011 particles 10-fold or more (data not shown).

Induction of antiviral state in different cell types

Some cell systems seem to acquire an antiviral state on stimulation with interferon inducers even though little or no interferon may be detected^{11,12}. Consequently, we tested for intracellular viral interference in cells in which the yield of interferon induced by DI-011 particles was abundant (primary chick embryo cells)³, low (human FS-4 cells)¹³ or nil (GMK-Vero cells)¹⁴. Monolayers of these three cell types were challenged with various multiplicities of DI-011 particles. After incubation for 20 h, the supernatant fluid was removed and assayed for its interferon content¹⁵. To establish the degree of intracellular viral interference, the cell monolayers were challenged with Sindbis virus ($m_{\text{FPF}} = 5$) in the presence of actinomycin D (1 $\mu\text{g ml}^{-1}$). The data in Table 2 reveal that DI-011 particles induce an heterologous antiviral state capable of reducing the yield of challenge Sindbis virus in chick embryo cells by 1,000-fold or more—even at the multiplicities of inducer particles which produce relatively low yields of interferon ($m_{\text{IFP}} > 2$). However, we note that the yield of interferon at these multiplicities, although low compared with the titres obtained at $m_{\text{IFP}} = 1$, is nonetheless significant (≈ 100 units ml^{-1}) and could itself account for development of an antiviral state during the 20-h test period. This consideration does not hold for human FS-4 cells, since little or no interferon was detected over the 100-fold range of multiplicities tested and illustrated in Table 2. Thus, DI-011 particles in human FS-4 cells produced an antiviral state in the apparent absence of released interferon. The heterologous antiviral effect (challenge with Sindbis virus) which develops in these human cells on infection with DI-011 particles indicates that the yield of interferon from cells is but one measure of DI-011 particle

Table 1 Factors affecting the induction of interferon by $\pm$ RNA DI-011 particles

Treatment of [$\pm$]RNA DI-011	Interferon yield (PR ₅₀ units per 2×10^7 cells)
Untreated (control)	37,000
Actinomycin D (1 $\mu\text{g ml}^{-1}$)	25
Cycloheximide (50 $\mu\text{g ml}^{-1}$)	< 20
VSV antiserum*	< 20
Heat (50 °C for 20 min)	33,000
Ultraviolet†	39,000

In all experiments an optimal multiplicity of DI-011 particles, that is, $m_{\text{IFP}} = 1$, was used to achieve maximal interferon yield as measured 24 h after infection. Actinomycin D or cycloheximide were added to the medium immediately after DI particle attachment and remained throughout the 24-h test period. The antiserum used during virus attachment could reduce the plaque-forming particle titre of a VSV Ind. stock by 99.9% in 30 min. The heat treatment reduced cell-killing, plaque-forming (and virion-transcription) activity each by 99% (ref. 4).

*Treatment with normal rabbit serum at a comparable dilution produced control (untreated) values of interferon.

†Figure 4 illustrates the exact relationship between ultraviolet dose and interferon induction by DI-011 particles, and compares it with the surviving activity for infectivity (plaque formation).

Table 2 Induction of intracellular interference against Sindbis virus by [$\pm$]RNA DI-011 particles in different cell types

Multiplicity (DI-011)	Sindbis virus yield (PFP ml ⁻¹)		
	Primary chick embryo cells	Human FS-4 cells	GMK-Vero cells
0	6.2×10^9	6.4×10^9	9.4×10^9
0.1	1.9×10^9	1.2×10^{10}	4.1×10^9
1	4.9×10^8	3.4×10^9	1.2×10^{10}
10	4.4×10^8	1.2×10^8	1.2×10^{10}
100	9.0×10^5	3.6×10^6	4.8×10^9

Monolayers of cells were infected with varying multiplicities of once gradient-purified [$\pm$]RNA DI-011 particles. Virus attachment was carried out for 30 min at 37.5 °C in attachment solution²⁸ containing 10 μ g ml⁻¹ DEAE-dextran. Monolayers were washed three times with warm medium and incubated at 40.5 °C (chick cells) or 37.5 °C (FS-4 or GMK-Vero cells) with 3 ml of medium per 60-mm dish. After 24 h, supernatant fluids were collected, processed, and assayed for interferon as described previously¹⁵. These monolayers were washed twice with attachment solution and infected with wild-type Sindbis virus at $m_{\text{PFP}} = 5$ –10. Actinomycin D (1 μ g ml⁻¹ for chick cells and 10 μ g ml⁻¹ for FS-4 and GMK-Vero cells) was included during virus attachment to prevent any further induction of interferon⁶. Cells were washed four times to remove unattached virus and 3 ml of medium was added to each plate. Infected monolayers were incubated at 37.5 °C for 10 h and the supernatant fluid titred by plaque assay for the Sindbis virus yield.

action on the cell; another is development of an intracellular antiviral state¹¹.

However, we have not ruled out the possibility that very small amounts of interferon are being produced¹², nor have we determined whether the reduced efficiency of inducing an antiviral state reflects a lowered probability of attaining a double-stranded configuration of [$\pm$] DI-011 RNA in FS-4 cells. The results with GMK-Vero cells are clear—no interferon is produced, nor is an antiviral state achieved by DI-011 particles in this cell which seems genotypically incapable of responding to inducers of interferon¹⁴.

Effect of [–]RNA defective interfering particles

Some standard preparations of DI particles might consist of both [–] and [$\pm$] (also [+]) type particles, and although [–]DI particles do not induce interferon (Fig. 1), they might interfere with, or enhance, the interferon-inducing capacity of [$\pm$]RNA particles. We tested this possibility by simultaneously infecting primary chick embryo cells with a multiplicity of DI-011 optimal for interferon induction ($m_{\text{IFP}} = 1$) and a high multiplicity of a conventional [–]DI-HR particle ($m_{\text{DIP}} = 50$). These large numbers of [–]RNA DI particles reduced the interferon-inducing capacity of DI-011 more than 10-fold, even though this multiplicity of [–]DI-HR particles had no adverse effect on the cells as judged by examination under phase contrast optics, or by counting single cells by the window technique¹⁶—confirming earlier observations that these particles are not intrinsically toxic^{3,8,9,17}. We are continuing to study this effect.

[$\pm$]RNA DI-011 particles are not intrinsically toxic

Because a single non-infectious particle of VSV—a defective cell-killing particle—suffices to kill a cell³, it is important to use highly purified populations of DI particles to test for their intrinsic toxicity^{3,8,9,17}. Preparations of [$\pm$]RNA DI-011 particles gradient-purified three times did not affect the plating efficiency of single GMK-Vero or chick embryo cells as tested up to $m_{\text{DIP}} = 50$, and so behaved like conventional [–]RNA DI particles³. Multiplicities in excess of 50 [$\pm$]RNA DI particles resulted in a measurable decrease in plating efficiency and the appearance of a cytopathic effect in monolayer cultures. It is not clear whether these effects reflect a cumulative toxic effect of these particles or the presence of otherwise non-detectable levels of infectious cell-killing particles. We suspect

the latter, since inclusion of VSV antiserum in the medium shortly after infection prevents the progressive cytopathic effect observed in monolayer cultures infected at $m_{\text{DIP}} > 50$.

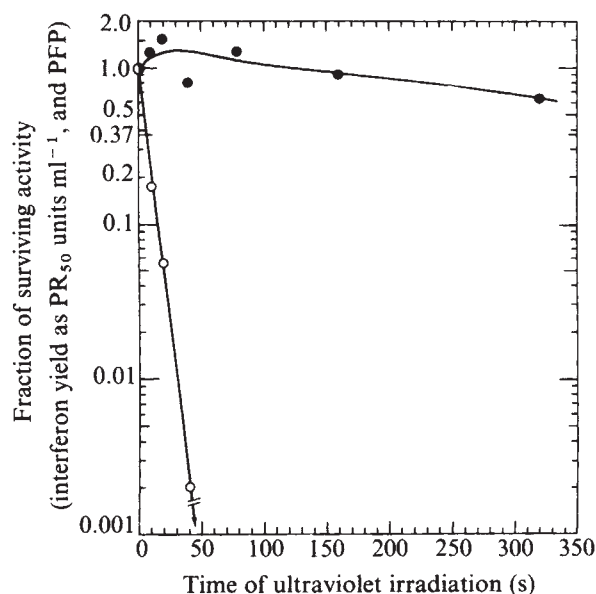
Single particle of [$\pm$]RNA DI-011 induces interferon

Our data indicate that a single [$\pm$]RNA DI-011 particle suffices to induce a quantum yield of interferon, and does not kill cells. The dose (multiplicity)–response (interferon yield) curve generated by DI-011 particles fits best a model where cells infected with a single particle (the $r = 1$ class of the Poisson distribution)¹⁰ synthesise and release a full yield of interferon, thus identifying a new attribute of these subvirions—their capacity to act as discrete interferon-inducing (IF) particles. The dose–response curve also indicates that cells infected with two or more IF particles respond differently and yield little or no interferon.

Requirement for dsRNA

We infer that interferon induction results from the action of a helical structure formed from some, if not all, of the complementary bases which make up the covalently linked [+]
message–[–]anti-message single-stranded RNA of the infecting DI-011 particle. In the same conditions, single DI-HR particles of conventional [–]RNA composition do not induce interferon. Thus, at an average of about 0.3 DI particle per cell, [+]
DI-011 and [–]
DI-HR induce the synthesis of approximately 25,000 and < 10 PR₅₀ (VSV) units of interferon respectively per 2×10^7 aged chick embryo cells⁶. That a double-stranded RNA formed intracellularly is the putative inducer of interferon in cells infected with DI-011 can also be inferred from the fact that [$\pm$]DI-011 and [–]
DI-HR particles both obtain polypeptides from viral transcripts synthesised by the coinfecting standard B particle of VSV¹⁸. R. Lazzarini has shown by double isotopic labelling and coelectrophoresis that the polypeptide composition of DI-011 is identical with that of the parental infectious type (personal communication). DI-011

Fig. 4 Interferon-inducing capacity of ultraviolet-irradiated DI-011 particles. Comparison with plaque-forming particle activity. One-half millilitre of thrice gradient-purified DI-011 particles and of wild-type VSV Ind. (strain MS) infectious B particles were added to separate 100-mm Petri dishes and exposed to a germicidal lamp (2,735 Å) which delivered 65.4 erg mm⁻² per 10 s. Samples of irradiated particles were tested either for interferon-inducing capacity (IFP, ●) as in Fig. 1, or for plaque-forming activity (PFP, ○).



and DI-HR particles apparently share identical polypeptides therefore, differing only in their content of covalently linked [+]-message-[-]-anti-message, and [-]-anti-message RNA, respectively. In addition, neutralisation of the interferon-inducing capacity of DI-011 preparations with specific antiserum to VSV (Indiana) demonstrates that the inducer molecule gains entry into the cell in a particle which possesses the specificity of the Indiana serotype, as do these DI-011 particles.

It will be difficult to prove directly the existence of a helical structure of [$\pm$]RNA at the level of the one molecule per cell presumed to form on interaction of a cell with a single particle of DI-011, particularly if once inside the cell, the number of base-pairs required for inducer function is as few as the six to twelve reported necessary for poly(rI)·poly(rC) to function similarly¹⁹. We do not know how the inducer molecule assumes a functional configuration in the cell, but it seems likely that in aged chick embryo cells (and perhaps to a lesser extent in human FS-4 cells) sufficient viral nucleoprotein is removed from the viral ribonucleoprotein core to permit a threshold amount of base pairing. Conceivably, only the [+]-stranded moiety of the [$\pm$]RNA functions as inducer. The high yield of interferon produced on infection with only a single DI-011 particle, presumably one molecule of [$\pm$]RNA, makes it, on a molar basis, and in terms of a delivery system, the most efficient inducer of interferon so far discovered.

The fact that the interferon-inducing capacity of the DI-011 particle is not affected by heat and ultraviolet is consistent with the [$\pm$]RNA's being the molecule responsible for interferon induction²⁰. We do not know yet whether a given molecule of [$\pm$]RNA from DI-011 can serve both as a template for replication, as in homotypic interference, and an inducer of interferon.

Possible role of [$\pm$]RNA particles in virus infection and disease

The remarkable efficiency of the [$\pm$]RNA DI-011 particle in inducing interferon and/or an antiviral state, coupled with the fact that interferon-mediated interference can prevent cell killing by VSV^{15,21}, suggests that the [$\pm$]RNA class of DI particle might play a part in persistent infection and viral disease beyond that presently postulated for its conventional [-]RNA counterpart². Involvement of the interferon system in persistent infection would provide a mechanism whereby cells can survive in the presence of lethal virus—an aspect not satisfactorily accounted for by present models. Thus, although a single DI particle can prevent completely the replication of its standard infectious helper virus^{3,8,9}, it does not prevent cell killing by that coinfecting virus³. Furthermore, we have demonstrated that single cells infected with an otherwise lethal dose of VSV, survive and form normal-sized colonies if, before infection, they have acquired an interferon-mediated antiviral state¹⁵. Current experiments provide evidence of a role for [$\pm$]RNA DI particles in persistent infection. They demonstrate that pretreatment of mouse L cells with [$\pm$]RNA DI-011 particles renders them refractory to cell killing by wild-type VSV, a property not shown by [-]RNA DI-HR particles

in the same conditions (M.J.S. and P.I.M., in preparation). We also note that the only DI particles of several types tested by Holland and Villarreal¹⁷ which are found consistently to establish persistent infection in BHK cells coinfecting with VSV, were those derived from stocks of *ts*-mutant G31(III). We have tested a preparation of long DI particles prepared from this mutant as supplied by E. Reichmann (obtained from J. Holland), and found that it acted as an excellent interferon inducer (data not shown). We also note that the presence, or generation of [$\pm$]RNA DI particles might account for the induction of interferon in mice by VSV²², or rabies virus (refs 23, 24 and references contained therein). We are now examining different types of DI particle for their IF particle activity, and by implication, for the presence of [$\pm$]RNA containing particles.

Characterisation of a closely related [$\pm$]RNA DI particle of VSV corroborates the existence of this new class of defective interfering particle²⁵. The ease with which single molecules of DI-011 [$\pm$]RNA can be delivered to the cell, and their efficiency at inducing interferon synthesis and/or an antiviral state makes them new and potent tools to define and understand the many effects of dsRNA on cells^{7,26,27}.

This research was aided partly by the Damon Runyon-Walter Winchell Cancer Fund, the National Science Foundation and National Cancer Institute, and benefited from the use of the Cell Culture Facility supported by the National Cancer Institute. We thank Drs L. Andrew Ball and Robert C. Warrington for their critical review of the manuscript.

Note added in proof: We have observed that the sparing of cells from the lethal action of VSV (and Newcastle disease or Sindbis virus) which develops upon pretreatment with DI-011 particles is obliterated completely when antiserum to interferon is present during the pretreatment. These results provide further evidence that the antiviral state induced by the DI-011 particle to heterologous viruses is interferon mediated.

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letters to nature

Q0000—398 is a high-redshift quasar with a large angular size

REDSHIFTS of between 2.5 and 3.1 have been measured for nine optically-selected quasars as part of the Cerro Tololo survey of the Southern Hemisphere (M. G. Smith, see ref. 1). None of these quasars were known to be radio sources. We

chose several accessible quasars from Smith's list as a starting point in our investigation to see if they might not be radio sources. We report that Q0000—398 is a quasar of high redshift and large angular size.

During October, 1976, we detected two of the quasars using the three-element interferometer of the National Radio Astronomy Observatory (NRAO), Green Bank, West Virginia,

Table 1 Data on quasars of considerable angular extent

Source name	1950.0 Radio position				Measured flux density				Redshift <i>z</i>	Angular extent θ (arc s)
	h	m	s		S_{2695} (Jy)					
Q0000—398A	00	00	29.78	(.4) —	39°49′ 27″.	(15)	0.013	}	2.827	134″
Q0000—398B	00	00	27.15	(.4) —	39 47 13.	(20)	0.015			
OE323A	03	13	46.19	(.4) +	34 26 27.8	(3.)	0.073	}	1.16	27.4
OE323B	03	13	44.38	(1.) +	34 26 31.1	(3.)	0.135			
M0758+120*	07	58	14.41	(.1) +	12 01 42.5	(.8)	0.08		2.66	see text
OS210A	16	06	10.74	(.1) +	28 56 43.1	(.2)	0.10	}	1.99	31.1
OS210B	16	06	09.34	(.1) +	28 57 06.1	(.2)	0.15			

*In Table 1 of Baldwin *et al.*⁶ the optical and radio positions are reversed.

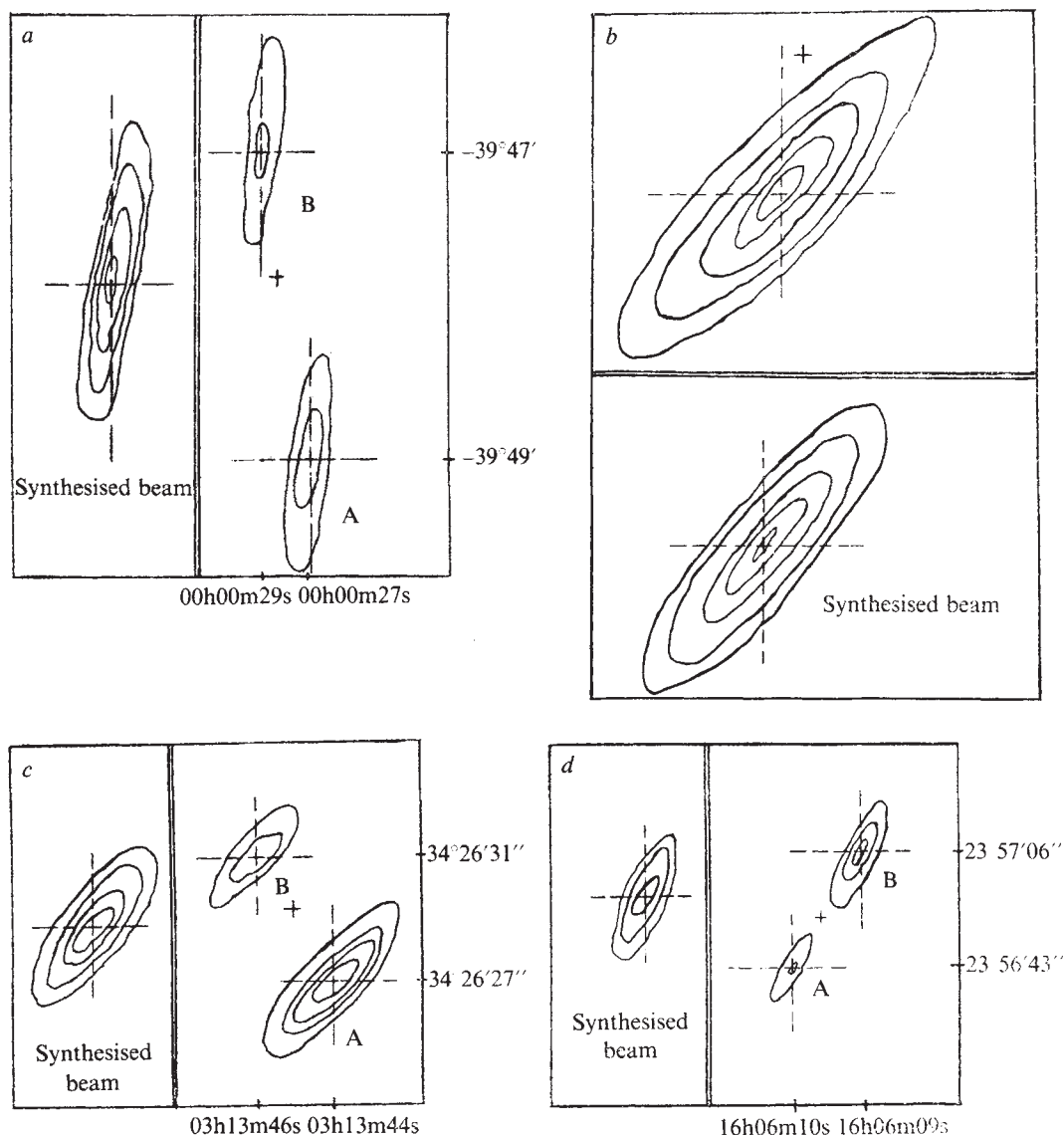
with a 300-1,200-1,500-m baseline configuration, operating at 2,695 MHz. The radio map for Q0000-398 (18 mag, $z = 2.827$) shows two weak components separated by 134 ± 40 arc s. If these components are associated with the optical object, Q0000-398 is the quasar with the largest known angular size at its redshift value. (Inspection of the Whiteoak extension

print of this area of sky shows no galaxies in this area, which may be emitting this radiation.)

The second quasar investigated, Q0049-393 (18 mag, $z = 2.94$) was also detected ($S_{2695} = 26$ mJy, where 1 mJy = 10^{-29} W m $^{-2}$ Hz $^{-1}$), but was unresolved.

These two quasars were observed along with several other

Fig. 1 Radio contour maps with synthesised beams at 2695 MHz for *a*, Q0000-398; *b*, M0758+120; *c*, OE323 and *d*, OS210. Crosses represent optical centroids. The angular separation of the radio and optical centroids for M0758+120 is about 14 arc s.



steep-spectrum quasars listed in Table 1. All of the sources were observed in ideal weather conditions with good hour angle coverage in the $u-v$ plane (allowing for the constraints of the elevation angle limit at low declinations, roughly $\pm 3^\circ$ at -39°). Details regarding the operation and characteristics of the interferometer are given in ref. 2.

Results for the quasars found to have considerable angular extent are presented in Table 1. Figures 1 *a-d* present clean maps at 2,695 MHz of four of the sources studied: Q0000-398, OE323, M0758+120, and OS210. Synthesised beams accompany each map. The radio component associated with M0758+120 is shifted by about 14 arc s from the optical counterpart.

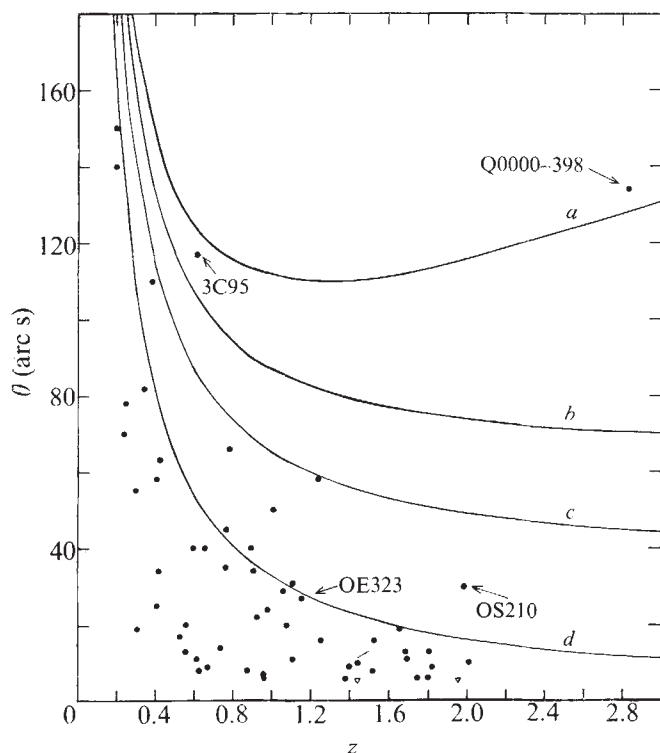


Fig. 2 Angular size-redshift diagram for 55 quasars with spectral indices $\alpha(408-1,415 \text{ MHz}) \geq 0.80$ and $\alpha(1,415-5,000 \text{ MHz}) > 0.60$. Curves *a-d* demonstrate the behaviour of a standard rod of 950 kpc in Einstein-de Sitter, Milne, de Sitter (steady state) and static Euclidean universes, respectively. Triangles represent upper limits. A Hubble constant of $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is assumed.

Further studies are required to determine if a fainter, weaker radio component is present, or if the radio source is actually associated with the quasar.

Assuming that quasar redshifts are cosmological, Friedmann models predict that quasars with constant linear dimensions will exhibit a minimum angular size as a function of redshift. The Einstein-de Sitter model, for example, with deceleration parameter, $q_0 = 1/2$, predicts a minimum near $z = 1.2$. Wardle and Miley³ have found that the maximum angular size of a sample of 166 quasars decreases with increasing redshift much faster than predicted by any conventional cosmological model. They conclude that evolutionary effects are responsible for the apparent Euclidean behaviour of the upper envelope to the angular size redshift diagram. The large angular extent of Q0000-398, however, tends to weaken this conclusion and suggests that a minimum may exist in the upper envelope function (see Fig. 2).

Additional evidence that the angular dimensions of quasars may exhibit a minimum is indicated by the results of Hewish *et al.*⁴. Their study of the angular sizes of compact components

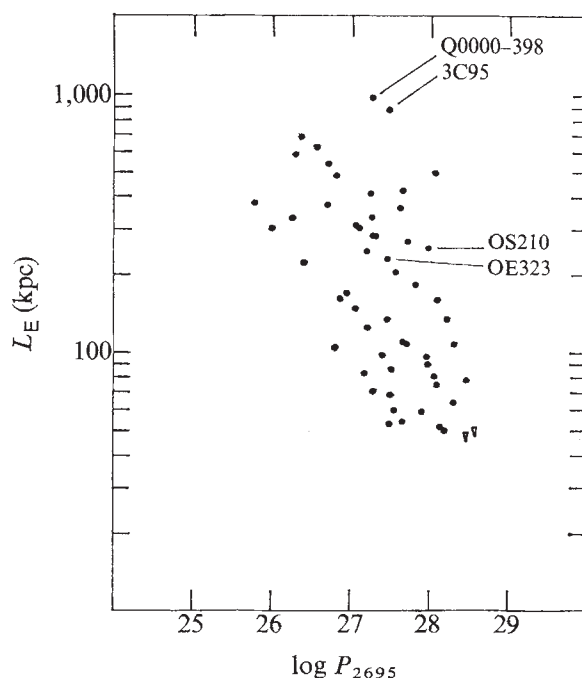


Fig. 3 Linear separation L_E (kpc) against 2,695 MHz spectral power (W Hz^{-1}) for Einstein-de Sitter model. Data are for the quasars in Fig. 2. Triangles represent upper limits.

of strongly scintillating 3CR sources shows evidence of the mean angular sizes increasing with higher redshift, as one would expect in evolving cosmological models with q_0 in the range of 0.5 to 2.0. Readhead and Hewish⁵ conclude that the apparent deficiency of small-diameter compact sources at large redshifts is real and significant.

Although Wardle and Miley³ find no evidence for radio selection effects as a result of the sample being selected by flux density, since Q0000-398 was selected optically and has a much lower flux density than most of the quasars in their sample, this suggests that radio selection effects may be important. A radio selection effect can arise from samples being selected by flux density if the luminosity of quasars decreases with increasing separation of components.

The largest angular-size quasars tend to have much steeper spectra than found for small-diameter quasars. Using the spectral-index criteria that $\alpha(408-1,415 \text{ MHz}) \geq 0.80$ and $\alpha(1,415-5,000 \text{ MHz}) > 0.60$ (where $S \propto \nu^{-\alpha}$) eliminates most small-diameter quasars as well as those with low-frequency cutoffs from the Wardle-Miley diagram. The remaining subset of 52 quasars, along with the other new data presented in this paper, is shown in Fig. 2. Figure 3 shows the relation between radio luminosity at 2,695 MHz and linear size for the quasars in Fig. 2. An Einstein-de Sitter model and a Hubble constant of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ has been assumed. The subset of 50 quasars shows an apparent inverse correlation (coefficient = -0.63 , significant at the 0.01 level) between luminosity and linear size (2 quasars with upper limit angular sizes were not included). No correlation between luminosity and linear size has been found for quasars having either spectral index flatter than the criteria set above. These quasars are more likely to possess self-absorbed components, which may smear out any relation between luminosity and linear size.

The results reported here demonstrate the importance of considering radio selection effects in the angular diameter-redshift diagram. Since any radio selection effects are removed when quasars are selected optically, more extensive mapping programs should be undertaken, looking specifically for large-scale structure around optically-selected, high- z , quasars.

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Implications of the Viking results for volatile outgassing from Earth and Mars

TUREKIAN and Clark¹ have recently discussed an inhomogeneous accumulation model for terrestrial planets, have compared the outgassing product from a veneer of carbonaceous chondrites with the abundances of volatiles on Earth and predicted the amounts of various gases for Venus. The recent measurements of rare gas abundances on Mars by Viking^{2–4} have prompted us to test such a model for Mars. We find that not only the relative abundance of H, C, N, ²⁰Ne, ³⁶Ar, Kr and Xe but their total amounts can be best explained if the outer layers of Earth and Mars were made of material similar to ordinary chondrites (LL) as suggested by Anders⁵, rather than carbonaceous chondrites as assumed by a number of authors in recent years.

In a non-homogeneous accretion model^{1,5,6} the planet is supposed to have accumulated from at least two different types of material. The accretion starts in a relatively hot solar nebula and therefore the inner part of the planet contains mostly high temperature condensates such as metallic iron, magnesium silicates, Ca–Al–Ti and rich oxides. As the planet grows, it is heated by the release of gravitational energy and if it is large enough (compare Earth and Venus) the proto planet becomes completely molten and liquid iron and nickel sink to the centre. With the passage of time both the proto planet and the solar nebula cool and the growing planet can then acquire a layer of low temperature condensates containing relatively large amounts of volatiles

crust and upper mantle, adopted from Turekian and Clark¹. The second line shows the abundances of these elements if they represent outgassing from carbonaceous chondrites (also from Turekian and Clark). Here we normalise the abundances for carbon. (Assuming 3.5% concentration by weight in the carbonaceous chondrites, complete outgassing from a 4-km layer of CCI would correspond to the abundance of carbon on Earth). Line 3 which represents the ratio of Earth to CCI shows that the agreement is far from satisfactory for the case of rare gases. Disparities of up to a factor of 37 exists for the case of ²⁰Ne.

A similar treatment using the volatile content of ordinary chondrites (LL) rather than CCI, produces a more satisfactory result. Line 4 is the total abundances of volatiles outgassed from a layer of ordinary chondrites, again normalised for carbon. Because of relatively low abundance of carbon (0.12%) in ordinary chondrites, line 4 represents outgassing products from a layer 116 km thick. The ratios shown in line 5 indicate a much better overall agreement for ordinary chondrites than for CCI (line 3).

Table 2 shows a similar treatment for Mars. Here of course there are more unknowns than on Earth, especially for C, N and H which may be in the surface layers rather than the atmosphere. We therefore follow a slightly different approach in constructing the table. Line 1 shows the abundances of rare gases, carbon and nitrogen measured in the atmosphere. Line 2 shows the amounts which would be expected if the outgassing is from the ordinary chondrite type material and normalised for ³⁶Ar. Line 3 then shows the amount of volatiles which may be in the surface layers of Mars or have escaped the planet.

The agreement for Kr and Xe is reasonably good. This leads us to presume that such an approach to the deduction of the total outgassing on Mars may be valid. If so, a number of inferences can be drawn from Table 2 as follows.

(1) The total volatile inventory on Mars corresponds to complete outgassing from an 0.5-km layer of ordinary chondrites or, if the layer is ~100 km thick (compare Earth), the outgassing has been only 0.5% effective.

(2) 145 g cm⁻² of C corresponds to ~500 g cm⁻² of CO₂. As only a very small amount of CO₂ is in the atmosphere, relatively large amounts (>90%) may be in the regolith, as proposed earlier by Huguenin⁷.

(3) 8 g cm⁻² of nitrogen either lies buried in the surface or has escaped the planet⁸.

(4) 66 g cm⁻² of H corresponds to ~600 g cm⁻² of H₂O.

Table 1 Volatiles on Earth, CCI and ordinary chondrites (g cm⁻²) and atom ratios normalised to carbon

	H	C	N	²⁰ Ne	³⁶ Ar	⁸⁴ Kr	¹³² Xe
Atmosphere, hydrosphere, crust, upper mantle (a)*	35,800	52,800	1955	1.3×10^{-2}	4.1×10^{-2}	2.0×10^{-3}	1.2×10^{-3}
Carbonaceous chondrites (CI) (b)†	34,300	(52,800)	4990	3.5×10^{-4}	2×10^{-3}	5.9×10^{-5}	7.6×10^{-5}
Ordinary chondrites (LL) (c)‡	24,200	(52,800)	3010	5.3×10^{-3}	3.6×10^{-2}	5.2×10^{-4}	6.4×10^{-1}
Atom ratios (a)/(b)	1.0	(1)	0.4	37	20	33	15
Atom ratios (a)/(c)	0.7	(1)	0.7	2.1	1.1	3.8	1.8

Hydrogen content data from LL ordinary chondrites taken as 0.05% by weight (Geiss, personal communication).

*From ref. 1.

†From van Schmus and Hayes and Mazor *et al.* quoted in ref. 1.

‡Data from ref. 15.

such as carbon, water, nitrogen and the rare gases. If the upper layers could then get heated again either by the radioactive decay of ⁴⁰K, ²³⁸U and ²³²Th or by some other mechanism, the volatiles contained in the low temperature condensates can be released to the surface of the planet. The suggestion is that this process was responsible for the formation of the atmosphere and oceans of the Earth.

Accepting this model in principle, we wish to discuss the nature of condensates which can most reasonably explain the volatile inventory on the surface of Earth and Mars.

Table 1 shows the total abundances of six volatile elements present in the Earth's atmosphere, hydrosphere,

Although the hydrogen content of the ordinary chondrite is the most poorly known piece of data in Table 2, it is interesting to note that the recent Viking results provide two interesting pieces of data which are relevant in this case. First, the surface layers of Mars seem to contain ~1% H₂O (ref. 9). If we assume that the regolith is ~100 m thick this would correspond to ~200 g cm⁻² of H₂O in the Martian surface. The second Viking observation relevant here is that the residual polar cap in the northern hemisphere is made of water ice¹⁰. Although there are large uncertainties in the estimates of ice thickness in the polar cap, our inventory of water outgassing on Mars would

Table 2 Martian volatile inventory derived by degassing from ordinary chondrites (g cm^{-2})

	H	C	N	^{20}Ne	^{36}Ar	^{84}Kr	^{132}Xe
Mars atmosphere observed	—	5.0	0.50	$< 10^{-4}$	1.0×10^{-4}	$\sim 6 \times 10^{-6}$	$\sim 1 \times 10^{-6}$
Ordinary chondrite (LL)	66	145	8.3	10^{-5}	1.0×10^{-4}	2×10^{-6}	$\sim 3 \times 10^{-6}$
Volatiles escaped or on surface	66	140	7.8	—	—	—	—

allow a thickness of up to 40 m, if escape of water from Mars can be ignored. If not¹¹, the polar cap thickness would be accordingly smaller.

(5) Major volatiles deduced from Table 2, H_2O , CO_2 , N_2 , add up to $\sim 1,100 \text{ g cm}^{-2}$. It has been suggested¹² that Mars goes through major climatic fluctuations and at times all volatiles could be in the atmosphere. According to our inventory the total mass of the atmosphere in such a case would be $\sim 1,100 \text{ g cm}^{-2}$ which, for Martian gravity, corresponds to $\sim 400 \text{ mbar}$; an atmosphere composed of $\sim 50\%$ CO_2 and $\sim 50\%$ H_2O with traces of nitrogen.

The ^{40}Ar degassing problem is a very different question than that of general outgassing of the planet (such as CO_2 , H_2O , rare gases and N_2). ^{40}Ar is a radiogenic product of ^{40}K and was nearly completely absent in the earlier stage of planetary formation (its degassing should be a function of its production and could be continuing at the present time (compare the Moon))¹³.

Viking measurements showed an $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the Martian atmosphere 10 times greater than the ratio in the Earth's atmosphere². Soil sample analyses¹⁴ give an upper limit for the potassium concentration at the surface of 0.25% (compared with the potassium concentration of 1.6% (ref. 1) in the Earth's crust).

We calculated the necessary thickness of a layer to account for the observed quantity of ^{40}Ar in the Martian atmosphere assuming two different values for the potassium concentration: 0.25% which is upper limit from Viking experiments and 0.08% which is the concentration in ordinary chondrites (LL). We find that layers of 2.5 km and 8 km respectively can account for the results of Viking measurements. These thicknesses are to be compared to the half-kilometre thickness calculated by us previously to account for the other volatiles (Table 2). This difference implies a different mechanism for outgassing of ^{40}Ar compared with the rest of Martian atmosphere.

The fact that the absolute amount of ^{40}Ar in the atmosphere of Mars could be explained by ~ 10 times less K in the upper layers of Mars than on the Earth, is interesting. It implies that the upper layers of Mars have not been substantially enriched in K and other radioactive elements perhaps because of the absence of extensive melting in the interior of Mars during its formation.

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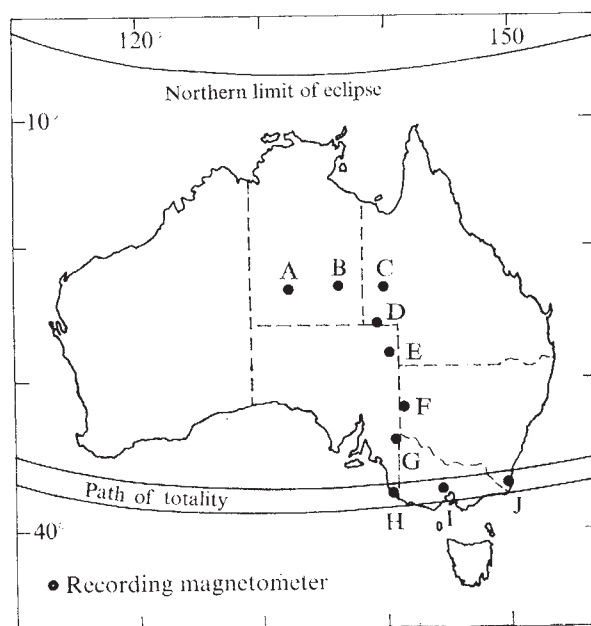
Magnetic observations of the solar eclipse of 23 October 1976 in Australia

SOLAR eclipses may cause geomagnetic effects by disturbing the flow of electric currents in the ionosphere^{1,2}, and a number of observations of this phenomenon have been made in recent years^{3,4}. To monitor any magnetic effects of the eclipse of 23 October 1976, we operated recording magnetometers at 10 sites in central and eastern Australia (Fig. 1); these instruments were in addition to the regular observatories of the Bureau of Mineral Resources near Perth and Melbourne. We present here a preliminary analysis of the declination variations recorded in the path of totality at stations H (Millicent, S.A.) and J (Merimbula, N.S.W.).

The recording instruments were Gough-Reitzel variometers⁵, set to take readings at intervals of 10 s. Because any eclipse effect was expected, on elementary grounds, to be mainly in the declination component, the declination sensors of the instruments were increased in sensitivity by a factor of four above their normal level. Timing of the 10-s readings (on film) was estimated to be accurate to within a few seconds.

The day of 23 October 1976 proved to be magnetically quiet in the declination component. Minor activity occurred at 0711 h Universal Time (UT), about 30 min after eclipse totality had passed. The declination records for stations H and J have been digitised at 10-s intervals for a 4-h period centred on the eclipse, normalised by calibration factors, and are shown plotted in Fig. 2.

Fig. 1 Sites of recording magnetometers operated to monitor the 23 October 1976 eclipse. Site H is Millicent Airfield (lat. $37^{\circ}35'\text{S}$, long. $140^{\circ}22'\text{E}$). Site J is Merimbula Airfield (lat. $36^{\circ}55'\text{S}$, long. $149^{\circ}53'\text{E}$).

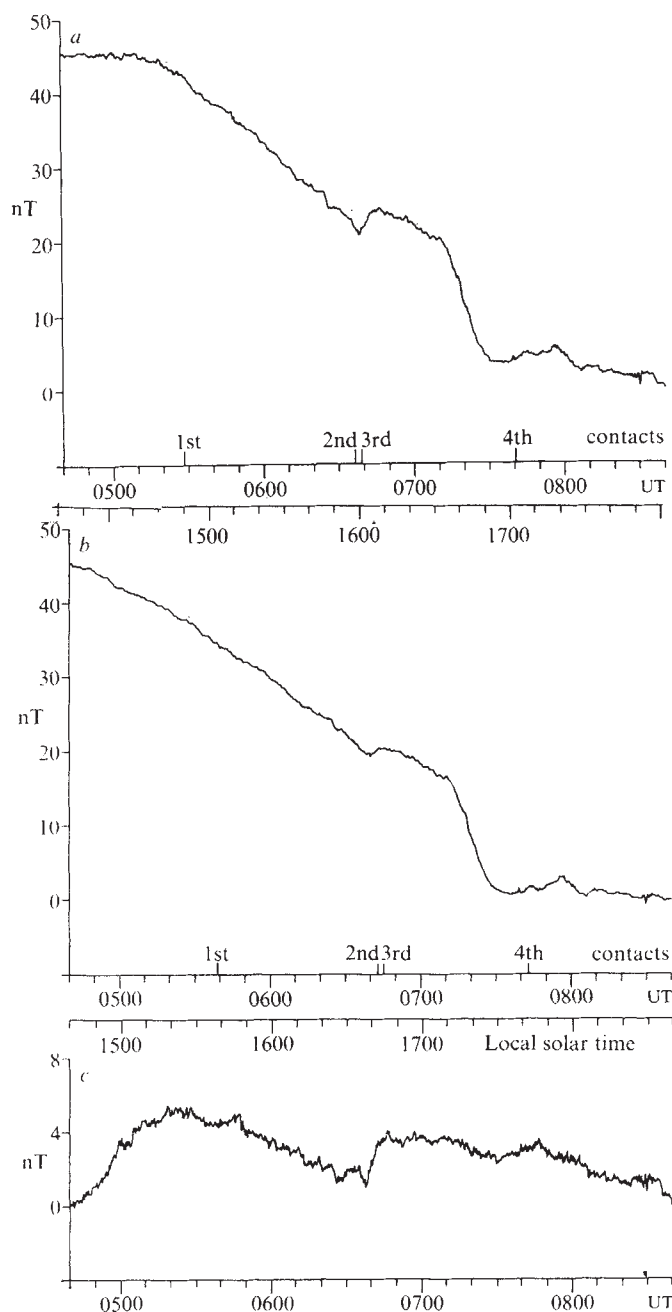


Also shown in Fig. 2 is the difference between them, subtracting trace J from trace H.

Natural electromagnetic induction in south-east Australia has been well studied by two previous magnetometer array experiments^{6,7}, which showed the coast effect to be strong in the vertical component of magnetic fluctuations, but to be negligible in the declination component. Thus, stations H and J were placed at opposite coastlines to achieve the greatest time difference between them for the passage of eclipse totality. The previous array studies have also shown that magnetic substorms and similar events occur near-simultaneously across the area, but that the quiet daily variation moves across with local solar time.

Thus, in Figs 2a and 2b one may expect to see: a background quiet daily variation, with station H lagging behind station J by 38 min; possibly some substorm activity occurring simultaneously at H and J; and an eclipse signal moving rapidly from H to J. In Fig.

Fig. 2 a, The declination record for site H. b, The declination record for site J. c, H-record minus J-record. All records are plotted relative to arbitrary zeros. Amplitude conversion factors are 6.4 nT per minute of arc for station H, and 6.8 nT per minute of arc for station J.



2c, where the traces are subtracted, the effect of the substorm activity should disappear to first order, and one may expect to see the daily variation subtracted from itself 38 min out of phase to give a smooth curve of amplitude approximately 5 nT, and some interference effect between the eclipse signals at H and J. It is significant that the activity starting at 0711 UT has indeed largely cancelled itself out in Fig. 2c.

The signal causing a depression in the declination traces at about 0638 UT is of interest as a possible eclipse effect, because: (1) it occurs at the time of the eclipse; (2) it does not cancel itself out in Fig. 2c as does the activity of 0711 UT; to be so strongly non-uniform it must be of local origin; and (3) it is of the correct sign, and order of magnitude, to be expected for the effect of an eclipse². Several aspects inconsistent with a simple eclipse model should be pointed out, however: (1) the signal at 0638 UT is not of the same strength at stations H and J; (2) it has not moved from west to east at the known rate of the centre of eclipse totality on the ground; and (3) it starts gradually but ends abruptly.

Digitisation of the records obtained at the other stations will be undertaken and a more comprehensive report is planned.

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Magnetic superleaks

Two new natural materials which behave as superleaks to superfluid ⁴He and also have magnetic properties are described here. In the search for magnetic superleaks the first choice was, obviously, a highly magnetic material such as a manufactured ferrite which has the advantage that its porosity is very uniform. We tested such a material but apparently not at a low enough temperature to be able to detect any onset point for the superfluid ⁴He. The pores or gap distances must be extremely small¹. Second, natural magnetic materials, among which the most obvious were different types of the so-called magnetites, were tested. Two magnetites of apparently quite different origin have been tested with superfluid ⁴He. One from the south-east of Argentina, Province of Rio Negro about 41°S latitude and about 30 km from the Atlantic Ocean (sample A) and another of unknown origin taken at random from the Mining Department of the University of Delft (sample B). An X-ray diffraction analysis of these two samples gave the following components: For sample A, haematite (Fe₂O₃) (ferromagnetic), magnetite (Fe₃O₄) (ferrimagnetic), mica and garnet [(Mg,Fe)₃Al₂(SiO₄)₃] (paramagnetic) and for sample B, mainly magnetite (Fe₃O₄) (ferrimagnetic) and chlorite, a sheet silicate [(Mg,Fe)₃AlSi₂O₁₀](OH)₂ (paramagnetic). Both samples are anisotropic aggregates and the magnetic characteristics are given at room temperature.

The method already described² to determine the onset temperatures of superleaks was used. A carbon sensor as a detector of the superfluid rests on the surface of a flat superleak inside a cell. When the superfluid through the superleak finally reaches the sensor, a noticeable jump in its resistance value is noted. This method has the advantage

over the capillary method that no direct observations of the liquid helium level are necessary, difficulties inherent in an optical cryostat.

The obtained onset temperatures are: for sample A 1.57 K and for sample B 1.72 K. Applying the well-known expressions³ of Ginzburg and Pitaevskii and of Mamaladze, the values for the gap width were obtained, respectively: for sample A, 36 Å and 24 Å and for sample B, 42 Å and 29 Å. Note that for superleaks made of natural materials the values of the onset temperatures are only approximate since they vary from different samples of the same material because of the non-uniformity of the diameters of the pores or gap widths. Also, with these measurements, there is a real doubt that the superleak is really a superleak. For instance, the way in which the superleak is fixed to the system, whether it is soldered to the glass of a cell or fixed with an indium wire gasket, might present microscopic leaks. Also it is impossible to apply any kind of reliable leak detection to a superleak. It is, however, possible to overcome that doubt with a reasonable number of observations and, as far as possible, mounting and dismounting the same sample to observe the reproducibility of the measurements.

The search for magnetic superleaks had its origin in the anisotropic magnetic superfluid phases of liquid ³He recently found. The application of magnetic superleaks to the peculiar anisotropic magnetic superfluid phases of liquid ³He at very low temperatures^{4,5} might be relevant. However, to check magnetic superleaks in the anisotropic superfluid phases of ³He will not be easy. In that physical situation those phases would be influenced by an adequately magnetised finite geometry medium, not necessarily those described here, which they filled, since below the transition temperatures the liquid has the properties of a superfluid and has magnetic properties, compared with those of nematic liquid crystals.

I thank Dr A. J. Leggett for valuable and encouraging remarks about the potential implications of a magnetic superleak in the superfluid phases of ³He. Thanks are given to Dr R. A. Kühnel for the X-ray diffraction analysis and to Mr J. G. Krom for helping with the measurements.

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Towards a theory of stochastic vorticity-augmentation

THE mechanism of genesis of atmospheric tornado-like vortices is not yet known, although the hydrodynamic mechanism of a mature tornado is reasonably well understood thanks to recent research efforts. This lack of understanding of the physics of tornado genesis has been a great handicap in attempts to avert tornado occurrences. I present here a new hypothesis to account for the formation of tornadoes.

The hypothesis is based on the observation that thunderstorm associated tornadoes almost invariably occur in the environment of an intensely (vertically) sheared horizontal wind, say $V_x(z)$ which is penetrated by a vertical columnar jet-draught along the z -axis. It is easily recognised that the vertically sheared wind field could serve as an abundant source of

ambient vorticity; nonetheless a casual theoretical consideration¹ of such a wind-jet interaction does not lead to a net gain of axial vorticity (ω_z) for the vertical jet at the expense of the horizontal vorticity ($\omega_y = -\partial V_x/\partial z$) according to the Helmholtz vorticity transport equation:

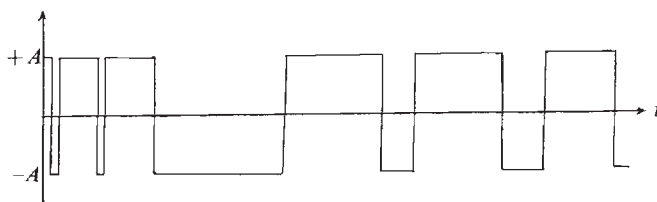
$$\frac{d\omega_z}{dt} = \omega_x \frac{\partial V_z}{\partial x} + \omega_y \frac{\partial V_z}{\partial y} + \omega_z \frac{\partial V_z}{\partial z} \approx \omega_y \frac{\partial V_z}{\partial y} \quad (1)$$

assuming $\partial/\partial z \ll \partial/\partial y$. A closer examination of the present wind-jet interaction, taking into account the resulting random lateral deflections of the jet caused by the alternating vortex sheddings in the wake of the cross-wind over the jet^{2,3}, shows that random sharp velocity gradients ($\partial V_x/\partial y$) are induced at the boundary of the random impulsively oscillating jet. This random input ($\omega_y \partial V_z/\partial y$) in equation (1) converts the latter into a stochastic differential equation having the axial vorticity (ω_z) as the output function. It is this stochastic vorticity transfer process that is considered herein as primarily responsible for starting a columnar vortex leading eventually to a mature tornado vortex after the pendant funnel extending down from the cloud base to the ground.

In this present study of the wind-jet interaction, I have constructed an extremely simplified model with only bare essential physics. For instance the entrainment which accounts for the incorporation of the ambient air into the jet is not taken into account. The hydrodynamic problem here is the determination of the probable increments of the jet axial vorticity due to the induced random input ($\omega_y \partial V_z/\partial y$) as a consequence of the random lateral displacement of the jet which can be viewed as a beam in Brownian motion⁴. Recognising the filamentary structure in a vortex jet⁵ and even in the fine scales of turbulent flows⁶, we envisage the jet as a bundle of vortex filaments along the jet axis (z) which faces a vertically sheared horizontal wind along the x -axis thus shedding alternating vortices on each side and, in so doing, inducing alternating hydrodynamic Magnus forces (along y -axis) on the jet. It can be shown that this many-filament system constitutes essentially a system of coupled nonlinear oscillators⁸, under the influence of a high-frequency harmonic forces, which therefore oscillates as though a random force were acting on it^{7,8}. The present many-body system is subdivided into a subsystem, namely a filament, and its environment. The influence of the environment on the subsystem is treated in a way similar that of a heat bath of statistical mechanics except that instead of a random force⁹ we have random vorticity impulse with stochastic properties supposedly given. In this way the equation governing the growth of axial vorticity for the total system is reduced to that for the subsystem alone with the quantity ω_z in equation (1) representing an unnormalised axial vorticity at a sample point (o) of the test filament; the quantity $(\omega_y \partial V_z/\partial y)_o$ an effective random vorticity impulses at the point o . We have thus a one-dimensional model neglecting the feedback and damping of the vortex-jet disturbances.

Consider a surface velocity gradient $(\partial V_x/\partial y)_o$ depicted by the simplest plausible stationary function known as the random telegraphic signals with values $+A$ and $-A$ alternately as shown in Fig. 1 where $A\alpha(t) = \omega_y(\partial V_x/\partial y)_o$ and $|\alpha(t)| = 1$. The probability of switching over the signal from one value to its

Fig. 1 Sample function of the $(+A, -A)$ random telegraph signal $A\alpha(t)$, $t > 0$



opposite is assumed to be governed by a Poisson distribution at a mean rate of $\mu \text{ s}^{-1}$.

The incremental axial vorticity can be obtained from equation (1):

$$\Delta\omega_z(\tau) = \omega_z(\tau) - \omega_z(0) = A \int_0^\tau \alpha(t) dt \quad (2)$$

The ensemble average of the squared incremental axial vorticity is given by¹⁰

$$\langle [\Delta\omega_z(\tau)]^2 \rangle = A^2 \tau \int_{-\infty}^{\infty} \langle \alpha(s)\alpha(s) \rangle ds \quad (3)$$

the integrand $\langle \alpha(s)\alpha(s) \rangle$ denotes the correlation function of the stationary random function $\alpha(t)$ and is equal to $\exp(-2\mu|s|)$. Thus:

$$\langle [\Delta\omega_z(\tau)]^2 \rangle = \omega_y^2 (\partial V_z / \partial y)_0^2 \tau \mu^{-1} \quad (4)$$

which gives the important parameter depicting the stochastic growth rate of the columnar axial vorticity of a vortex filament of the jet.

The more detailed probabilistic information is given by the probability $P(\Delta\omega_z, t)d(\Delta\omega_z)$ that the increment $\Delta\omega_z$ at time t lies between $\Delta\omega_z$ and $\Delta\omega_z + d(\Delta\omega_z)$. Recognising the Markovian nature of the stochastic process herein, we can determine the probability density $P(\Delta\omega_z, t)$ by solving its governing equation in the form of a driftless Fokker-Planck equation for diffusion⁹ and obtain the Gaussian distribution:

$$P(\Delta\omega_z, t) = (4\pi Dt)^{-1/2} \exp[-(\Delta\omega_z)^2/4Dt] \quad (5)$$

where $D = \langle (\Delta\omega_z)^2 \rangle / 2t$. If a critical value for $\Delta\omega_z$ is prescribed, say $(\Delta\omega_z)^* = \beta \sqrt{\langle (\Delta\omega_z)^2 \rangle}$, for large β ,

$$P[\Delta\omega_z > (\Delta\omega_z)^*] \simeq (2\pi)^{-1/2} \beta^{-1} \exp(-\beta^2/2) \quad (6)$$

In conclusion, a new cause of vorticity transfer between an ambient sheared-wind and a transverse-penetrating jet is proposed for which an elementary one-dimensional theory is formulated. It points out the relevant quantities to be determined, theoretically or experimentally, in describing the present stochastic mode of vorticity-augmentation.

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Theoretical evidence for metastable cyclic ozone

OZONE is a vital component of the atmosphere. Knowledge of its properties is important to the understanding of its involvement in chemical and photo-induced reactions which can affect the stability of the ozone concentration in the upper atmosphere. The reactivity of ozone and its unusually low thermochemical stability with respect to partial dissociation are difficult to reconcile with an apparently multiply bonded

symmetric bent structure. This has led to speculation^{1,2} concerning alternative forms of ozone such as the equilateral triangular cyclic form (isoelectronic with cyclopropane) from which facile partial dissociation could be envisioned. To have an important role such a form would presumably need to be thermally accessible from the ground state and bound with respect to partial dissociation. The spectroscopic structure determination of the symmetric bent ground state³⁻¹⁰ has been finalised¹⁰ (r_e (O-O) 127.1 pm, angle 116.8°), and there was recently experimental confirmation^{11,12} of excited states of this species^{13-16,22} which are bound with respect to dissociation, including the predicted ³B₂ state^{13-17,22}. Investigations of bound excited states which are stable to partial dissociation have been stimulated by the sequential observation during O₂ radiolysis experiments^{12,18,19} of two short-lived 'ozone precursors' O₃^a and O₃^b following recombination of ground state O₂ and O. O₃^b may be vibrationally excited ground state ozone²⁰, whereas the O₃^a precursor which shows absorption¹² at ~ 315 nm in the ultraviolet (and possibly²¹ the emission observed at 1500 cm⁻¹ in the infrared) has not yet been given an identity beyond an apparent lifetime of 5 μs. The species showing the single infrared emission²¹ is interesting as D_{3h} cyclic ozone is expected to show one (degenerate) infrared active fundamental, fewer than any alternative structure. Theoretical evidence for the stability of cyclic ozone has been conflicting. In 1973 Wright¹ calculated it to be 26 kJ mol⁻¹ more stable than the ground state; in 1972 and 1975 Hay *et al.*^{13,22} calculated it to be less stable by ~ 150 kJ mol⁻¹ (that is, above partial dissociation energy $\Delta H^\circ = 101$ kJ mol⁻¹) and in 1974 Shih *et al.*¹⁴ have concluded it is 63 kJ mol⁻¹ above the ground state. Shih *et al.*¹⁴ have recognised that considerable uncertainty still exists in their computation on total and relative energies, and their calculated ground state structural parameters differ significantly from experiment. We have reinvestigated the relative stability of cyclic ozone and symmetric bent ozone and have obtained a total energy for the ground state of -224.7710 hartree which is 471 kJ mol⁻¹ lower than the previous best model wavefunction of Hay *et al.*¹³ who predicted cyclic ozone to be above dissociation. In other words, the relative shortcoming in total energy in all previous computations on each conformer has been around five times the key energy difference between ground state ozone and the partial dissociation products, $\Delta H^\circ = 101$ kJ mol⁻¹. The computed optimum geometric parameters (bond length $r_e = 126.4$ pm and bond angle $\alpha_e = 117.3^\circ$) are sufficiently close to the experimental values to lend some confidence to our model wavefunctions in regard to structural predictions. The corresponding wavefunction for cyclic ozone has an energy expectation of -224.7637 hartree at an optimum bond length of $r_e = 143.5$ pm (bond angle 60°). On this evidence the difference in the energy of the local minima on the ozone ground state conformation hypersurface is less than one fifth of the partial dissociation energy. We, therefore, confidently predict the existence of a bound species of ozone having an equilateral triangle conformation. We believe this species may be the O₃^a precursor, which in the experiments referred to^{18,19} is presumably vibrationally excited.

Our disagreement with Hay *et al.*¹³ regarding the stability of the cyclic conformer has a number of origins. Hay *et al.* seem not to have optimised molecular geometries using their best bases and their best correlated wavefunctions, and have apparently omitted polarisation basis functions in any study of the cyclic conformer. The basis sets we used are comparable to the best previously used at the Hartree Fock level E_{HF} (bent, SCF optimised geometry) - 224.314 hartree, E_{HF} (bent, CEPA optimised geometry) - 224.3058 hartree and E_{HF} (cyclic, CEPA optimised geometry) - 224.3011 hartree. The significantly lower final conformational energies we have obtained despite the fact that our basis is not designed to obtain inner 'atomic' correlation effects is due to the use of a pair natural orbital (PNO) basis which is designed to optimise the configuration expansion convergence. We used ~ 750 PNO con-

figurations in our final calculations on each conformer, which is equivalent to $1-2 \times 10^4$ canonical configurations based on HF orbitals.

Our computer modelling of the wavefunction of each ozone species was intended to reduce previous basis set and geometry-optimisation limitations, taking the following course:

(1) Initial optimised geometries for each species were obtained from a survey of the surface (1014 points)²³ using simple independent particle SCF wavefunctions based on atomic STO-3G basis augmented with sets of single component gaussian bond functions between each pair of oxygens (3 sets). The bond function sets comprised one *s* gaussian and three *p* gaussians all of which were single gaussian fits to corresponding Slater functions of exponent $\xi = 1.1$. These bond functions afforded some valence deformation and polarization.

(2) With these geometries, large and flexible atomic oxygen basis $\{9s, 5p\} \rightarrow [5, 1, 1, 1, 1; 3, 2]$ in standard notation; ref. 24) were situated at each nucleus, and three equivalent sets of mid-bond bond functions for each species were optimised at the SCF level by varying the characteristic Slater exponent for the sets (ξ_{opt} (ground state) = 1.87, ξ_{opt} (cyclic) = 1.95).

(3) With the partially optimised set of 45 expansion functions so obtained (data to be published, P.G.B.) for each species, the valence SCF molecular orbitals were subject to a localisation procedure and the many-body effects of electron correlation among the valence electrons was estimated by following the CEPA-PNO procedure of Meyer²⁵ and Ahlrichs *et al.*²⁶. At this stage correlation interaction between pairs only belonging to the same atom as well as the intrapair correlation were taken into account (yielding 57 sets of pair natural orbitals).

(4) Using the incomplete CEPA-PNO wavefunctions, geometric parameters were reoptimised (small changes resulted) yielding the values quoted above.

(5) Full valence CEPA-PNO correlation energy corrections were obtained for each reoptimised geometry species (including 81 sets of pair natural orbitals) yielding the respective energies for the ground state and cyclic ozone quoted above. The CEPA-PNO energy difference of 19.2 kJ mol⁻¹ favouring symmetric bent ozone is paralleled by the corresponding PNO-CI^{25,26} difference calculated by us of 15.8 kJ mol⁻¹.

We believe that limitations in our model due to incomplete basis sets and neglect of core and core-valence correlation will affect our computations of relative energies by less than 20 kJ mol⁻¹, and that further refinement of the wavefunctions for each species will not affect our prediction of the existence and stability of cyclic ozone.

We expect cyclic ozone to be involved in ozone reaction kinetics and atmosphere chemistry where inclusion of its role may improve atmospheric modelling²⁷. In interstellar gas clouds such a metastable species might be expected to be trapped by slow collisional deactivation. The absence of a permanent dipole moment, however, is against the usual methods of detection of pure rotational transitions which would provide the best confirmation of the identity of cyclic ozone.

We are working to calculate the spectroscopic and other properties of cyclic ozone.

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High absorption efficiency for ingested plutonium in crabs

ALTHOUGH marine organisms have been strongly implicated in the biogeochemical cycle of plutonium, there is disagreement as to whether they can assimilate it efficiently¹⁻⁷. We have examined the fate of plutonium in a single, worm-crab food chain after ingestion of the isotope which had been metabolically incorporated into the predator's food. Contrary to absorption efficiencies of tenths to hundredths of 1% derived from studies with vertebrates⁸⁻¹⁰, our results show that relatively large fractions of plutonium are readily absorbed from ingested food and incorporated into the predator's tissues.

The radioisotope ²³⁷Pu was used in all experiments. The advantages of using ²³⁷Pu tracer to study environmental kinetics of ^{239,240}Pu have been discussed by others^{5,11}. The relatively high specific activity of this isotope facilitates the use of amounts of plutonium similar to the levels found in the environment. Tracer solutions were prepared by adding sufficient ²³⁷Pu in either the +4 or +6 oxidation state to 50 ml cotton-filtered seawater to give a concentration of approximately 8 nCi ml⁻¹. Polychaete worms (*Nereis diversicolor*) were exposed to labelled seawater for several days, thoroughly rinsed and whole-body counted for ²³⁷Pu activity as described previously⁵. In the first series of experiments six individual shore crabs (*Carcinus maenas*) received two to three contaminated *Nereis* and were whole-body counted immediately, replaced in their individual aquaria with running seawater ($\approx 18^\circ\text{C}$) and given sufficient unlabelled worms to ingest throughout the experiment. The crabs were whole-body counted periodically to measure excretion of the radionuclide, and at the same time their faecal pellets were filtered from the water and monitored for ²³⁷Pu to assess defaecation as a route of elimination. At prescribed times, individuals were dissected to show the distribution of residual ²³⁷Pu. In a second series of experiments, two large edible crabs (*Cancer pagurus*) ingested four separate rations of labelled worms within 48 h and were subsequently maintained for 3 weeks in conditions similar to those already described. The size of *Cancer* prohibited whole-body counting and the elimination was estimated from the knowledge of ²³⁷Pu ingested, lost by defaecation and retained in the various tissues.

Figure 1 shows the plutonium whole-body elimination patterns displayed by *Carcinus*. The initial rapid loss noted during the first 2-4 d represented gut clearance of unassimilated plutonium. This was verified by analysis of faecal pellets; during this period approximately 90% of whole-body loss occurred through defaecation. The fraction of total loss due to defaecation then decreased markedly from 90 to 40%, suggesting the increasing importance of soluble excretion processes. This period corresponded to the phase

during which whole-body elimination proceeded at a much slower, nearly exponential rate (solid lines in Fig. 1). This phase represents loss of that fraction of plutonium absorbed from the food; extrapolation of this component to day 0 facilitates estimation of absorption efficiency. The high values for the six individuals, from about 20–60%, were not expected in view of results from previously published vertebrate studies^{8–10}, and we are unaware of comparable data for invertebrates.

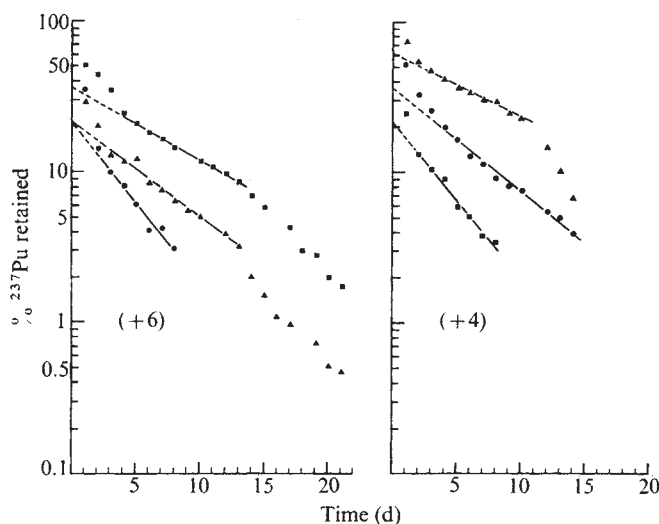


Fig. 1 Elimination of ^{237}Pu after single ingestion of labelled worms by six individual *Carcinus*. Assimilation coefficients for Pu were estimated by resolving the different components of the loss curves using standard mathematical methods routinely used in radioecological studies^{12–15}. The loss curves are treated as a two component elimination system described by the expression $A_t = A_0(a_1 e^{-k_1 t} + a_2 e^{-k_2 t})$, where A_t is the whole-body radioactivity at time t , and A_0 is the initial whole-body radioactivity immediately following ingestion of labelled food. a_1 represents the fraction of unassimilated ^{237}Pu eliminated at a rate k_1 ; a_2 is that fraction of assimilated ^{237}Pu eliminated at rate k_2 ; a_2 (absorption efficiency in our study) is determined graphically by extrapolation of the linear part of the long component (solid line) to time zero. Enhanced elimination of the assimilated ^{237}Pu noted in three individuals after about 12–14 days is assumed to be a reflection of increased metabolic rate probably associated with a given stage of the intermolt cycle; therefore these data were not included when fitting the longer component by a least squares estimate.

Dissections indicated that the plutonium absorbed from the food had become distributed within the crabs. In most cases the largest fraction (43–85%) was in the hepatopancreas which serves as the digestive gland. Lesser amounts were in other tissues such as shell (8–43%), and in muscle and gill (5–10%). ^{237}Pu was at or below the detection limit in stomach, gut and haemolymph. In the second experiment a larger crab, *Cancer*, was given more ^{237}Pu to facilitate measurement. Because of the experimental design it was not possible to estimate absorption efficiency; however, it was undoubtedly high, for 12 and 41% of ingested +6 and +4 ^{237}Pu , respectively, was retained in the tissues at the time of dissection. The relative distribution of the radionuclide among the tissues of *Cancer* generally confirmed that noted with the shore crabs.

Concentrations (c.p.m. per g wet mass) of residual ^{237}Pu in both crabs were by far the highest in hepatopancreas, with less in gill, stomach, shell and muscle. Certain trace elements¹⁶ and radionuclides¹⁷ are absorbed from ingested food and can accumulate to high levels in crustacean hepatopancreas; similar mechanisms seem to operate in the initial absorption of the large fraction of plutonium removed from the crab's food during passage through the gut. The low concentration of Pu in muscle relative to the other tissues was similar to that found in crabs and lobsters

which either had been contaminated in the environment^{2,18} or had accumulated the radionuclide directly from water in laboratory studies¹⁹.

Reports^{1,7,20} have emphasised the need for more information on transuranic transfer coefficients between various links in marine food chains to predict total amounts potentially available to man. We have examined this aspect in a simple, two-step invertebrate food chain and have found that Pu naturally incorporated into food can be assimilated into the predator's tissues with efficiencies several orders of magnitude higher than those characteristic of mammalian systems. The difference in absorption efficiencies between mammals and marine crustaceans is most likely a result of differences in digestive physiology; it is, however, conceivable that the way the radionuclide was administered influenced the degree of assimilation. Although only data for crabs are reported here, similar experiments carried out in our laboratory suggest that high plutonium absorption efficiency is also common to other marine invertebrates. Thus, it is possible that data indicating extremely low gastrointestinal absorption of plutonium by vertebrates are not applicable to species comprising the bulk of the marine biomass.

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When is sex environmentally determined?

THERE are several sex determining mechanisms which produce two sexes in a population (dioecy or gonochorism)^{1–5}. In many of these, the offspring's sex is determined at or before conception, as in male or female heterogamety. In several organisms, however, the offspring's sex is determined later than conception, by some environmental influence upon the offspring. We consider here why these environmental mechanisms have been maintained; that is, why have they not been replaced with an alternative system that determines sex at conception? We propose that labile sex determination (not fixed at conception) is favoured by natural selection when an individual's fitness (as a male or female) is

strongly influenced by environmental conditions and where the individual has little control over which environment it will experience. Our argument also applies to sex expression in hermaphrodites^{6,7}.

The basis of a theory of labile sex determination lies with two principles, first noted by Fisher⁸, who applied them to sex ratio evolution: (1) both sexes contribute autosomal genes equally to the next generation, that is, everyone has a mother and a father, and (2) the fitness of an individual is measured relative to others of the same sex. Relative fitness is the ratio of an individual's actual fitness (survival $\times$ fertility) to the average fitness in that sex and must account for the relative abundances of both sexes. Selection thus favours the ability of a parent or offspring to manipulate the sex ratio to result in offspring with the highest fitness in each sex⁹.

To illustrate, if an individual finds itself in an environment where it can become a below average female or an above average male, selection will favour its becoming male because it can pass on more of its genes than if it were female. Selection may therefore favour 'environmental sex determination' (ESD) because of the control it allows an individual. When sex is determined at conception, the individual no longer has this control but can begin 'developing into' its sex immediately. Early development of sex allows an individual to become a better male or female and is probably the major advantage of genotypic sex determination.

Selection should most strongly favour ESD when (1) the offspring enters an environment, away from the parent, which has a large effect on its lifetime fitness; this environment is patchy, some patches conferring more of an advantage to females than to males and vice versa; and (2) the offspring and parent have little control (or predictive ability) over which patch type the offspring enters. These conditions make it unfavourable to determine sex at conception because of the possibility that a male will enter a patch that is much more favourable for a female, or the reverse.

There are several ways in which an environment may be 'patchy' relative to the selective value of being a male or a female. (1) There is local competition for mates. If an area has a high concentration of females and a low concentration of males, then an individual enjoys the greatest success when it is a male. (2) Resources may have differential value to males or females, and they may be patchy in distribution. If an offspring must garnish most of its resources in a restricted environment, and if there is a sex difference in the advantage of the resource, then the offspring should become the sex which benefits the most under the circumstances. Resources which may have differential value to sex function include sunlight (in plants) and other nutrients for growth (a large size is often more advantageous to one sex than the other). (3) Predation (or other mortality sources) may be patchy and could be sex specific.

The inability of the parent or offspring to choose a patch type also has a major role in favouring ESD. If an offspring can choose its patch, sex can be determined at conception and the offspring enters the patch that will give its sex the highest fitness. Inability to choose patch type will often be correlated with immobility; however, parental mobility does not preclude ESD. If parents deposit offspring in (or offspring enter) environments from which they cannot escape during some crucial stage of development, selection may favour an environmental mechanism when the parent or offspring is unable to evaluate the patch type prior to entry. In contrast, if the parent can evaluate the patch (but the offspring cannot leave it), selection may favour parental ability to match the sex of the offspring to the appropriate patch. Such mechanisms occur among parasitic Hymenoptera^{10,11}.

Environmental sex determination is known in several plants and animals, and these seem to fulfil the expected characteristics.

Many species of nematodes which parasitise insects (family Mermithidae) and some which parasitise plants (genera *Meloidogyne* and *Heterodera*) are believed to have ESD^{2,12-14}. The life histories are basically alike: young (eggs) are either deposited on a host or they locate a host and enter. The larvae attain almost adult size while in the host. Adults (at least males) leave and mate outside the host^{12,15}.

The sex of the nematode is determined after entering the host, and sex ratio varies with parasite density inside the host. High

density results in an abundance of males, whereas low density results in mostly females^{2,12-14}. Large size is apparently more important to female than to male fitness (females are universally the larger sex in nematodes). Thus a juvenile in a crowded host will not be able to attain a large size; it can become an average male or a below average female: selection should favour it becoming the male. In uncrowded hosts, an individual gains more as a female.

Among marine invertebrates, ESD is known in *Bonellia* (an echiurid), and several parasitic isopods (for example, *Stegophryxus* and *Ione*)^{2,3,16}. These organisms usually have dwarf males which live on the female. Larvae are planktonic and adults are immobile (*Bonellia*) or are restricted to the host. Larvae that settle alone become females, those that attach to females become males. In these, the resource of strongest sex-dependent value is the female, because of the ability of the male to remain small, live upon her and fertilise her eggs. The example of parasitic males partially violates the condition that the organism be unable to choose patch type. A larva that encounters a female can probably choose to avoid settling on her. Despite this, the absence of choice still largely applies because all larvae presumably do not encounter females (or do not have the choice to). Therefore, a larva that differentiated into a male too early would not be guaranteed to encounter a female.

Sex may also be environmentally determined in monostolid crustaceans¹². These parasitic copepods have life cycles similar to the nematodes discussed above in that the adults are free and mobile, and most of the larval growth takes place inside the host. As in nematodes, crowding in the host results in a preponderance of males.

Among higher plants, there are numerous examples of dioecious species where some individuals change sex between seasons of reproductive activity^{4,5,17}; this provides evidence for the widespread occurrence of ESD in a group where it was previously given very little attention^{18,19}. Also, there are many cases where male and female plants occupy different habitats, suggesting ESD or perhaps differential mortality by sex¹⁷.

Several well-documented examples of plants with ESD are orchids of the genera *Cynoches* and *Catasetum*^{20,21}. Sunlight seems to be the resource which is differentially beneficial. Plants grown in bright sunlight become female, those grown in shade become male. Also, larger plants tend to be female. It is not clear why sunlight is more important to femaleness than to maleness (why should it be more important to seed production than pollen production?), but the pattern is well documented.

The arguments have been thus far directed towards maleness against femaleness in dioecious species. Natural selection should also favour the ability of a hermaphrodite living in a patchy environment to alter its allocation of resources between male and female function⁷.

A sequential hermaphrodite should exercise control over the time at which it changes sex in accord with the above idea. As fitness of the second sex is often size dependent, it should often change sex according to size rather than age. For example, if an individual is reduced in size just before the expected size of sex change, it should remain the first sex until recuperated from the perturbation. Examples of this are well documented: in a polychaete (*Ophryotrocha puerilis*)² and a plant (*Arisaema triphyllum*)^{4,5,22,23}. Other examples of labile sex change, related to male against female fitness, are also known in fish (the labrid, *Labroides dimidiatus*)²⁴ and some marine gastropods (for example, *Crepidula*)^{2,25}.

In a simultaneous hermaphrodite, selection should favour the ability to alter the ratio of sperm (pollen) to ova (seeds) produced in response to environmental conditions⁷ (as simultaneous hermaphroditism is commonly associated with immobility in the adult stage)⁶. Examples seem to be scanty, but the reallocation in monoecious plants due to factors such as nutrition or sunlight is well documented^{4,5,26,27} (C. Smith, personal communication), particularly in the Russian literature²⁸.

Interestingly, this suggests that one selective advantage of monoecy in plants is that it allows the use of hormonal systems to control male against female allocation. This alteration is as simple

as changing the ratio of male to female flowers. Supporting this idea is the observation that populations consisting of males, females and monoecious individuals are among the most common of all types of mixed populations in plants²⁹. We interpret the males or females as hermaphrodites who have reallocated resources to one or the other sex. Such lability would seem to be less possible in plants with hermaphroditic flowers.

As this paper was being written we became aware that similar ideas were being developed in four other labs: K. E. Hoagland with protandrous molluscs; C. Smith for monoecious trees; L. Gilbert and M. Condon for protandrous vines; and C. Freeman, K. Harper and L. Klikoff for dioecious plants. We acknowledge their independent thought. We thank G. C. Williams, R. Shine, J. Cranford, J. Werren, N. Negus, P. Berger, G. H. Orrians, J. Maynard Smith and Thomas Gibson for cooperation. This work was supported by a grant from the PHS.

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Monogamy and sex change by aggressive dominance in coral reef fish

In several sequentially hermaphroditic coral reef fish, of which individuals first function as females and then males (protogynous hermaphroditism), dominant males can control production of other males by aggressive dominance over females^{1–4}. Robertson suggested that socially controlled protogynous sex changes might operate only in species with a well defined polygynous social system “based on individual relationships”^{2,3}. Further exogenous and endogenous factors may control sex change in species which school anonymously and in which the individuals lack special individual relationships. We describe here socially controlled proteandric hermaphroditism (individuals functioning first as males and then as females) in the anemone fish *Amphiprion*, in which females control production of females by aggressive dominance over males.

Amphiprion is widely distributed in the tropical and subtropical Indopacific region and is symbiotic with several sea anemones⁵. A 38-month field study of the ecology and social behaviour of *A. bicinctus* in Eilat, Israel (Red Sea) and a 2-month study of *A. akallopis* at Aldabra indicated that the breeding population of both consisted of large functional females with smaller functional males (Table 1). Gonadal examinations of 170 *A. akallopis* and 41 *A.*

Table 1 Mean and standard deviation of body length and wet body weight of the 10 largest males and females of *A. akallopis* and *A. bicinctus*

	<i>A. akallopis</i>		<i>A. bicinctus</i>	
	Male	Female	Male	Female
Length (mm)	73±3	97±5	113±7	129±6
Weight (g)	7.1±1.1	19.5±2.9	28.0±3.9	46.1±7.6

bicinctus showed that both species are born as males and females, but during ontogeny undergo functional male then female phases. Immature oocytes are always present in the testes of functional males (Fig. 1b), while functional females show no sign of testicular tissue.

The typical social unit is a large female, a single smaller male and a varying number of subadults and juveniles, none of which is offspring of the adult pair. Both species

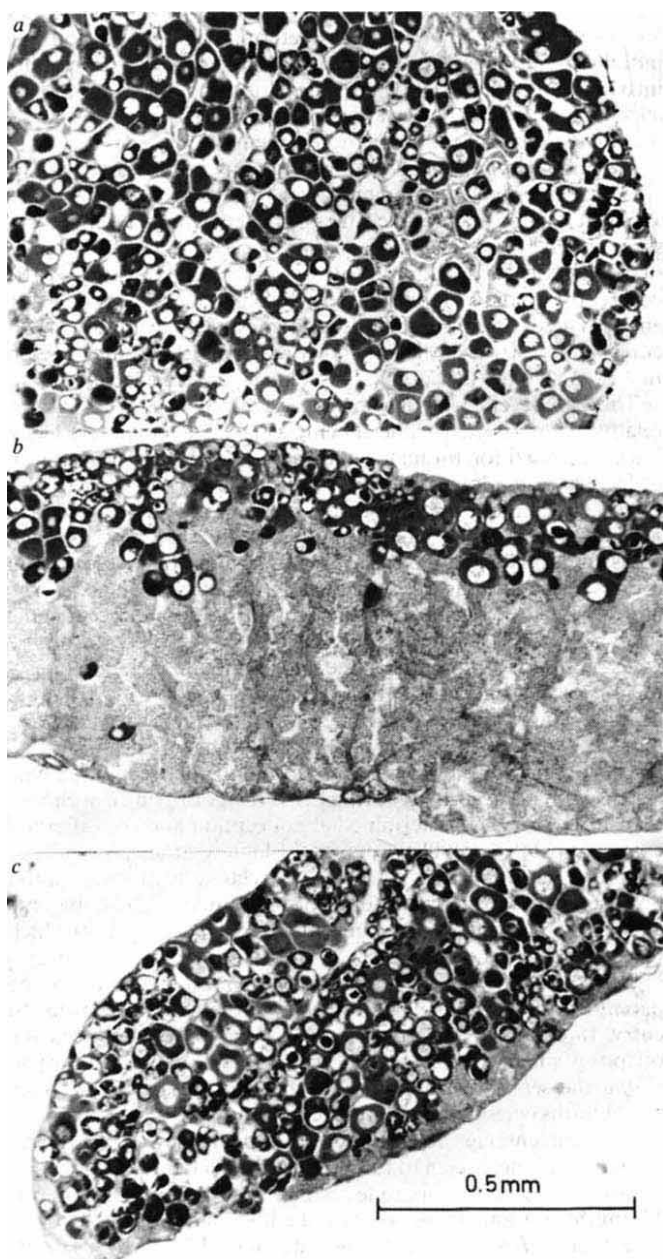


Fig. 1 Development of the testes of *A. akallopis* according to the social status of the male. Males of approximately the same body length were selected. a, Previous beta-male (65 mm) 63 d after removal of the dominant female; b, β -male (70 mm) in functional state; c, γ -male (68 mm), development of mature testicular tissue suppressed.

live in closed monogamous groups based on individual recognition and individual pair bonding^{6,7}. Several pairs of *A. bicinctus* remained on their home anemone for 3 yr without changing partners. Differences in group size result from differences in the ecology of occupied anemones (Table 2). Occasionally groups of up to 10 adult *A. akallopisos* are found on a single anemone colony. The average group size of *Amphiprion*, however, is restricted by the dimensions of the anemones to no more than two adults. "Monogamy by force" and permanent pair formation is favoured under these ecological limitations⁷. The largest and oldest individual of a group is always female and dominates the male, subadults and juveniles (Fig. 2). Subadult males are attacked by both sexes, the dominant alpha-female and high ranking males (Fig. 2). The beta-male particularly attacks the sexually competing gamma-male. In two groups of four *A. akallopisos* each, 53% of 861 and 64% of 559 observed, intragroup aggressive acts were performed by beta against gamma. The largest male prevents other males from spawning, thereby maintaining a monogamous social structure. The intragroup social pressure determines the gonadal development of the subdominants. Their testes are smaller and show little or no mature testicular tissue. Low ranking males are psychophysiologically castrated.

Sex reversal occurs in different social situations. After removal of a dominant female in an aquarium group of five mature *A. akallopisos*, the beta-male took the alpha-position and changed sex in less than 63 d (Fig. 1a). Sex change was also induced in field experiments by the removal of females from 24 pairs of *A. bicinctus*; six of the 24 males remained solitary while the remaining 18, joined by subadults of unknown origin, turned into females. The first of these females laid eggs after 26 d. To demonstrate the influence of dominance on sex change, different sized males were paired forcibly on empty anemones in the reef, and the gonads of six pairs were examined after 2 months and another 10 pairs after 6 months. The largest and dominant males always changed to females. Sex reversal also occurred in the absence of intragroup social pressure. Twelve functional males placed separately in the sea, six caged and six free, after 2 months showed various degrees of increased oocyte production. The uncaged males were more advanced towards becoming female in contrast to the caged males which were probably more stressed. Sex change was much slower in both experimental groups compared with dominant males which were paired forcibly with smaller males in the same conditions. The reversal of sex change from female to male could not be induced experimentally. Forcible pairing of functional females ($n=7$) resulted in death or severe injury to the subdominants. This study demonstrates socially controlled sex change by aggressive dominance. Females control the procreation of other females. They restrict the size of the breeding population and actively suppress males which are likely candidates for future females.

If *Amphiprion* were gonochoristic (1:1 sex ratio), the chances for homosexual and heterosexual pairings resulting

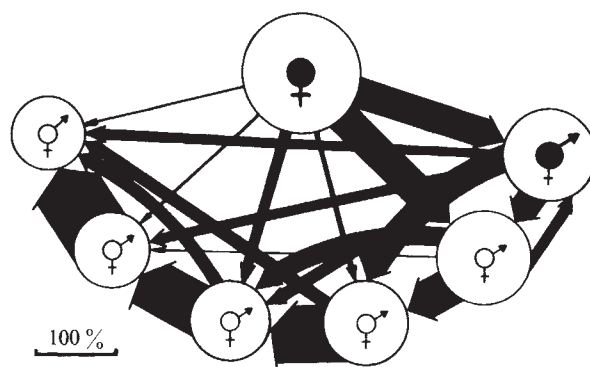


Fig. 2 Rank order by aggressive dominance in a group of seven *A. akallopisos*. An aggressive interaction is defined as a single chase, bite or frontal display. The relative body size of each individual is expressed as the square of the circle. The sex sign indicates the sex determination of the individual. A full circle denotes a functional, reproductive individual. The direction of the big arrows points to the receiver of the aggressive act. The distribution of aggressive acts of each individual is expressed as a percentage of total aggressive acts (= diameter of the arrow) which are performed by one individual against other group members. 752 intragroup aggressive acts were recorded.

from randomly arriving larvae or juveniles or migrating adults would be equally distributed. Redistribution of sexually mismatched individuals would presumably result in migration of one of the individuals. Migration, however, increases the risk of predation. Furthermore, low population density and unpredictable distribution of the host reduces the chances of finding an anemone or an anemone with a partner of the opposite sex. These risks can be alleviated by socially controlled hermaphroditism where the largest and dominant animal controls the sex of the subdominants. In the case of death of a pair partner, this partner is easily replaced: the female through a fast sex change of the dominant male, the male from the stock of subadults or juveniles which arrive or live together with the adult pair. Juveniles are stunted but grow rapidly when an adult dies^{3,7}. Furthermore, their ambisexual nature⁸ allows formation of pairs by dominance when no adults are present. The young fish in the proximity of the breeding fish are always an insurance of potential males and females. Socially controlled sex change enables recruitment of breeding pairs independent of the sex of arriving young fish and without the necessity of leaving the host in search of a mate. Functional mates are produced whenever they are needed. It seems to be advantageous for a female to be larger than the male of the pair. A small *Amphiprion*-male can successfully fertilise and guard the eggs of a big female. Size increase of the male would not necessarily increase the reproductive success of the male. On the other hand, a larger female can produce more eggs than a smaller one (body weight and weight of ovaries are correlated in both species, *A. akallopisos* $r = 0.55$ ($P < 0.001$), *A. bicinctus*

Table 2 Comparison of both fish species and their host anemones

Species	Host anemones	Life style of host	No. of observed hosts	No. of adult fish per host	No. of juvenile fish per host	% Hosts occupied
<i>A. akallopisos</i>	<i>Radianthus ritteri</i>	Colonial	82 Colonies	2.6	0.6	100
<i>A. bicinctus</i>	<i>Gyrostoma helianthus</i>	Solitary	228	1.1	0.3	99.2
	<i>G. quadricolor</i>	Solitary	122	—	0.9	69.7
	<i>R. koseirensis</i>					

The data for *A. akallopisos* were taken along transects in the lagoon channels of Pass Femme and Pass du Bois at Aldabra; for *A. bicinctus* in different reef zonations of the Nature Reserve in Eilat.

$r=0.45$ ($P<0.05$). Therefore, the female of a monogamous pair benefits in reproduction by an increase in size. This could determine the direction of sex change.

A system of monogamy and sex change by aggressive dominance of females with them controlling the breeding population seems to be adaptive in ecological conditions which restrict group size to an average of not more than two adults. Probably the system evolved in response to the low population density and unpredictable, opportunistic distribution of the host anemones which occur in nearly all reef zonations from shallow to deeper waters.

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Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex

STUDIES of the polytene chromosomes of the mosquitoes of the *Anopheles gambiae* complex have revealed fixed rearrangements based on paracentric inversions between all the six sibling species and inversion polymorphisms at the intraspecific level¹⁻⁵. Inversion polymorphisms involve mainly chromosome arm 2R and are particularly frequent in the species formerly designated as A and B for which the names *An. gambiae* Giles, 1902 and *An. arabiensis* Patton, 1905 have been proposed⁶. These are the most ecologically adaptable members of the complex and the most important medically⁷. They are sympatric over the greater part of the African savannas. We report here on relationships between the behaviour of these mosquitoes and their inversion polymorphism.

Investigations were conducted in the Garki district (Kano State, Nigeria), a typical Sudan Savanna area located north-east of Kano where *An. arabiensis* generally outnumbers *An. gambiae* and breeding of both species is strictly seasonal, depending on rainfall. The main working hypothesis was that of a differential probability of contact with man and/or his domestic environment for the mosquitoes carriers of different inversion karyotypes. The availability of suitable polytene chromosomes in the adult ovarian nurse cells⁸ enabled us to test this hypothesis directly by comparing inversion frequencies in female mosquitoes collected on the same day and in the same locality (a village in the Garki district) but in different situations (for example, indoors and outdoors). Preliminary evidence of non-random distribution of *An. arabiensis* with different 2R arrangements^{9,10}, obtained in 1972 and 1973, stimulated further comparative sampling in 1974 and 1975.

The 2R chromosome polymorphism of *An. arabiensis* from the Garki district involves six common paracentric inversions (Fig. 1). This complex system of overlapping and independent contiguous inversions permits no less than 15 different whole

arm arrangements (2R, 2Rb, 2Rbc, 2Rd, 2Rbd, 2Rbcd, 2Ra, 2Rab, 2Rabc, 2Rad, 2Rabd, 2Rabcd, 2Rbe, 2Rabe, 2Rbf), the frequencies of which cannot always be estimated because some of the 105 heterozygous combinations result in karyotypes which are very difficult to distinguish from one another (for example, 2Ra/2Rbcd, 2Rabc/2Rd, 2Rad/2Rbc, 2R/2Rabcd). Variation in 2R was therefore studied in terms of three sectional standards with the corresponding independent inversion

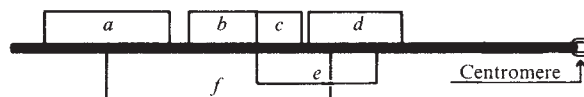


Fig. 1 Diagrammatic representation of 2R chromosome inversions in *An. arabiensis* from the Garki district. Inversion pairs b-c and b-e are interpreted as cases of extreme contiguity with one break point apparently coincident. The available evidence indicates that both c and e are based on 2Rb as the inverted arrangements so far recorded are 2Rb, 2Rbc and 2Rbe, but not 2Rc nor 2Re. Note that, in absence of 2Rb, both arrangements 2Rbc and 2Rbe could be interpreted as due to tandem inversions based on 2R. Inversion f is based on 2Rb producing the 2Rbf arrangement which overlaps with all other 2R inversions.

systems +^a ↔ a, +^b ↔ b ↔ bc and +^d ↔ d and with two other overlapping inversions, be overlapping the last two systems and bf overlapping all three. As might be expected from close linkage existing between the independent inversions, obvious departures from random association were observed; these will be analysed separately.

Table 1 compares the basic 2R chromosome variability in samples of *An. arabiensis* females collected in the village of Jirima during the 1973-1975 rainy seasons. The mosquitoes collected were: biting outdoors on donkeys (CD), biting outdoors on men (CM-OUT), biting indoors on men (CM-IN), resting indoors after blood meal (IR) and blood fed in exit trap (ET). Consistently significant differences were observed between these samples in the a and b inversion systems. The arrangements +^a and bc were more frequent in CD, CM-OUT and ET samples than in the corresponding CM-IN and/or IR samples indicating that the mosquitoes with these chromosome types have a lower propensity to bite and rest indoors. In other words, these data point to a relationship between the 2R inversion polymorphism and differential endophily and endophagy. The substantial similarity of the chromosomal constitution of CD and CM-OUT samples indicates that differences in zoophily compared with anthropophily are not involved.

The 2R inversion polymorphism seems typically flexible (in the sense defined by Dobzhansky) as the alternative arrangements show wide seasonal and geographical changes in frequency with fairly obvious correlations with climatic and ecological conditions (Coluzzi *et al.*, unpublished). Thus those 2R arrangements which are more frequent in Jirima in outdoor samples of *An. arabiensis* (+^a and bc) increase in frequency in southern more humid areas (Guinea Savanna), while those more frequent in Jirima in indoor samples (a and b) increase in frequency in northern more arid zones (Sahel Savanna). Data so far available for *An. gambiae* are strikingly similar. These findings suggest that the differential endophily is primarily an expression of differential climatic adaptations. Assuming a close integration of physiological and ethological responses to environmental heterogeneities, genetical variants adapted to an arid climate would be more prone to bite and rest indoors as the indoor environment generally shows a higher nocturnal saturation deficit than the outdoor environment.

Relationships between behavioural variations and inversion polymorphism have been demonstrated in the laboratory in *Drosophila* (see the review by Parsons¹¹) and in *Anopheles*¹²⁻¹⁵. Our demonstration of such relationships in nature emphasises their evolutionary importance as well as their practical significance when dealing with a vector species. The practical implications are obvious when it is considered that a differential endophily and endophagy such as that recorded in *An. arabiensis*

Table 1 Frequencies (%) of 2R chromosome arrangements in samples of *Anopheles arabiensis* females obtained in the village of Jirima (Garki district, Kano State) during the 1973–75 rainy seasons

Collection method	N	+ ^a	a	+ ^b	b	bc	+ ^d	d	be	bf
5 collections from 24.8 to 12.9, 1973										
CD	858	42.31	56.06	8.51	46.15	30.42	69.93	15.15	13.29	1.63
IR	246	30.49	68.70	9.76	54.88	21.95	67.48	19.11	12.60	0.81
χ^2		10.672†	12.096‡	0.232	5.485*	6.315*	0.431	1.936	0.030	0.416
4 collections from 31.7 to 9.8, 1974										
CD	722	48.89	48.61	6.37	49.31	31.72	75.35	12.05	10.11	2.49
IR	422	33.65	63.27	9.48	51.42	24.88	72.04	13.74	11.14	3.08
χ^2		24.591‡	22.443‡	3.266	0.395	5.694*	1.352	0.546	0.200	0.161
7 collections from 31.7 to 16.8, 1974										
ET	186	45.16	52.15	4.30	49.46	32.26	72.04	13.98	11.29	2.69
IR	772	33.81	62.05	8.16	50.78	24.22	68.91	14.25	12.69	4.15
χ^2		7.898†	5.717*	2.716	0.058	4.646*	0.554	0.0005	0.158	0.509
4 collections from 1.9 to 9.9, 1975										
CD	2262	40.19	56.45	5.97	46.86	30.59	70.51	12.91	13.22	3.36
CM OUT	1628	42.32	53.93	5.84	47.79	30.65	71.38	12.90	11.98	3.75
CM IN	780	34.23	64.23	6.92	52.56	25.26	71.79	12.95	13.72	1.54
IR	1132	31.63	65.28	8.13	51.06	23.94	69.61	13.52	13.78	3.09
χ^2		41.347‡	49.903‡	7.415	10.788*	24.063‡	1.490	0.294	2.546	8.814*

Sampling procedures used were: (1) Collection on donkeys outdoors (CD): mosquitoes were captured on 10–14 animals scattered in the village and exposed all the night. (2) Collection on men outdoors (CM-OUT): mosquitoes were captured on eight individuals from various parts of the village who had been exposed all the night. (3) Exit trap samples (ET): collections were made at 0600 h from 15 exit traps left on doors all the night. (4) Collection on men indoors (CM-IN): mosquitoes were captured on eight individuals exposed in closed huts (four per hut) all the night. (5) Indoor resting samples (IR): early morning (0600–0800 h) collections were made in 8–15 human habitations (huts) by pyrethrum spray capture or hand capture. The mosquitoes obtained from animal and human baits (mostly partly fed or unfed) were provided with a full blood meal on donkey during the same night. Blood fed specimens from all samples were kept in 200 ml paper cups (no more than 30 per cup) in favourable climatic conditions until 1400–1600 h and then processed for chromosomal examination either directly or after preservation in Carnoy fixative. No significant mortality was recorded during the maintenance period and more than 90% of the chromosome preparations were generally favourable for inversion reading.

*, †, ‡ Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

in the Garki district is expected to produce variations in vectorial efficiency and in the response to house spraying with insecticides. Some of the available data suggest that inversion polymorphism constitutes the basic genetical variability from which behavioural resistance to the insecticide may develop. Entomological investigations carried out by the WHO in the Garki district revealed important heterogeneities between *An. gambiae* s.l. from various villages, consistent with a positive association between exophily and residual density after insecticide (propoxur) spraying¹⁸. Such positive association clearly extends to the pre-spraying frequency of the 2R chromosome arrangements favouring exophily and exophagy in both *An. arabiensis* and *An. gambiae* (unpublished data).

With respect to evolutionary implications the non-random distribution of carriers of different arrangements implies some kind of ecotypic divergence among them, as suggested by Mayr¹⁷ and supported by the work of Dobzhansky *et al.* in *Drosophila pseudoobscura*¹⁸. Thus the maintenance of inversion polymorphism could be based, at least in part, on annidation, in the sense defined by Ludwig¹⁹. A further consequence of non-random distribution of carriers of different arrangements might be their differential probability of shift to new or marginal ecological niches. This would mean a differential probability of forming isolates in which genetic reorganisation can be initiated, thus indicating the possibility of a causal involvement of inversions in speciation^{20–22}.

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Control of plant respiration through non-photosynthetic light action

THE effects of photosynthetically active light on carbon loss by plants are attracting much attention^{1,2}, but light action through mechanisms other than photosynthesis, though demonstrated as early as 1954 (ref. 3), is usually ignored in discussions of metabolic inefficiencies. Nevertheless, dim red light acting through phytochrome can clearly regulate the time course of CO₂ output in axenic cultures of *Lemna perpusilla*. The precise pattern of output under a given light schedule depends on the nitrogen source supplied; on nitrate or ammonium, the main daily peak seems to be timed in the same manner as some aspects of the photoperiodic control of flowering^{4–6}. Essentially parallel results on O₂ uptake are reported here, indicating that the phenomena in question are respiratory in a reasonably strict sense.

Recording small changes in O₂ against the high background in air is essentially impossible over periods of days with methods currently available. Hence the intermittent-flow principle developed by Bruce and Street⁷ for the detection of insects in

stored grain was used. The change in gas concentration developed between periods of flow moves to the analyser as a 'bolus' when flow is resumed, producing a signal greater than that attainable otherwise. Comparing data so obtained with the earlier CO_2 results might be misleading, however, as intermittent flow might modify the patterns in some way. Thus all

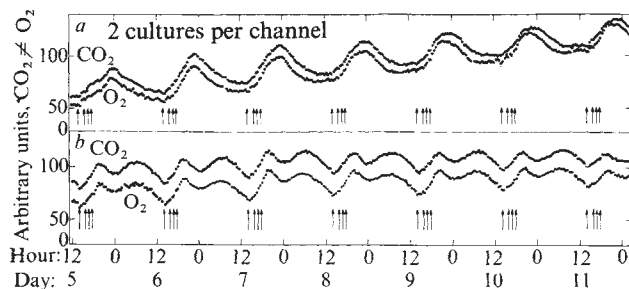


Fig. 1 Rates of O_2 uptake and CO_2 output by cultures on nitrate or aspartate as sole N sources and entrained to 4-h skeleton main light periods. Points are at 0.5-h intervals, each being assigned to the hour or half-hour immediately preceding its occurrence. The clone used was originally obtained from Dr Elias Landolt as *Lemna perpusilla* 6746, but it has also been described as *L. paucicostata*²³. Stocks were maintained under continuous cool white fluorescent light (about 3,200 lx) at 25–27 °C, on a complete mineral medium supplemented with sucrose, yeast extract, tryptone and EDTA (refs 5, 6). Experimental cultures established by transferring batches of fronds from stock cultures to the media being tested were then capped for connection to the air streams, all substantially as in ref. 5 except that 125-ml Erlenmeyer flasks with 50 ml of medium were used instead of bottles. Nitrogen-free Z medium supplemented with either aspartic acid (a) or KNO_3 (b) at 15 mM was used, with the aspartate medium sterilised by filtration rather than autoclaving. As before, brief exposures to dim red light (cool white fluorescent screened with Rohm and Haas red 2444 Plexiglas to yield 10–35 $\mu\text{W cm}^{-2}$ at plant level) were used to simulate main light periods. In the results above, 15-min exposures starting at 1400, 1545, 1645 and 1745 h daily, local time (arrows), represent a 4-h main light period; exposures at 1400, 1715, 2030 and 2345 h would simulate a 10-h main light period⁶. Constant temperature in the experiment shown was about 27 °C. Cultures contained 0.2–1.4 g fresh weight of plant during the experiment, and were held in conditions being tested for 5 d before connection to the intermittent-flow gas analysis system. Each blank and sample channel received, in succession, 4 min of air flow at 50 ml min^{-1} followed by 26 min without flow. The recorded analyser outputs obtained show the samples as spikes separated by the blank values. The sample-blank differences plotted above thus represent the rates of change in O_2 or CO_2 in relative units. A cold trap (–18 °C) was interposed before the analysers to prevent water vapour from reaching them. Oxygen was determined by magnetic susceptibility using a Beckman G-2 oxygen analyser with a reversed range, nominally 21.0–20.5% (5,000 p.p.m. full scale). The infrared CO_2 analyser, a LIRA Model 303 (Mine Safety Appliances Co.) also had a full-scale range of 5,000 p.p.m. Given the intermittent-flow mode, the actual relationship between the arbitrary O_2 and CO_2 units plotted is uncertain, and will of course vary depending on the relative drifts of the two analysers. Rough calculations suggest that culture size would have to be increased 60- to 100-fold in order to obtain comparably useful data from, for example the automatic recording respirometer described by Dilley *et al.*²⁴: a detailed description of the present system and an assessment of its quantitative aspects will be presented elsewhere (Hillman and Tempel, in preparation). The timing of each peak can be estimated by inspection, or objectively, by summing the times of the eight or more highest values during the period in question and dividing by the total number of values, in accord with the following rules. The time of the highest value, or the times of the several highest (values of rank α) are taken first. To these are added the times of all values one unit less (rank β) and then of all of ranks γ and lower, as necessary, until at least eight values have been summed; but if one value of any given rank is included, then all others of that rank not separated from it by two or more unused lower values are also included. As an example, the peak time for the O_2 values on day 10 on nitrate, above, combines the values at 0600, 0630 and 0730 h (rank α), those at 0530, 0700 and 0800 h (rank β) and those at 0400, 0430, 0500, 0830 and 0900 h (rank γ) for a total of 11 values giving a peak time of 6.50 h after midnight, which is 16.5 h after the start of the light period, or 12.5 h after the start of the dark period.

channels were also monitored with a CO_2 analyser. The first apparatus constructed had one sample channel and two blanks (without plants), and the second, two samples and three blanks. Most of the results considered are from the second version.

Figure 1 summarises a typical experiment under 4-h skeleton light periods on aspartate and nitrate media. The courses of O_2 uptake and CO_2 output are essentially parallel throughout, while the patterns on the two media differ. On aspartate, there is one peak per day and on nitrate, two; earlier work with CO_2 output led to the designation of the second daily peak on nitrate as the main peak.

The time of each main peak was estimated in both the experiments shown in Fig. 1 and another performed the same way. The means and standard errors calculated from the combined experiments place the peaks in O_2 uptake on aspartate and nitrate at 7.9 ± 0.32 and 16.1 ± 0.18 h, respectively, after the start of each light period. The corresponding peaks in CO_2 output occur at 8.1 ± 0.35 and 16.6 ± 0.18 h, times not significantly different from those for O_2 . All these values, as well as the patterns themselves, are essentially those obtained earlier for CO_2 output monitored by continuous flow^{5,6}.

In the CO_2 output data referred to, changing the length of the main light period shifts the main peak on nitrate medium. The line relating the time during the dark period at which that peak occurs to the length of the light period has a slope of -0.425 ± 0.049 for skeleton main light periods from 4–10 h long⁶. That is, lengthening the light period by 1 h causes the peak to occur slightly less than 0.5 h earlier, as measured from the start of the dark period, which is a delay of slightly more than 0.5 h from the start of the light period. To determine whether the same relationship holds for O_2 uptake, time-course data were obtained on nitrate medium under schedules with 4- and 10-h skeleton light periods in the same experiment. For the 6 d recorded, the mean time of the main peaks under the 4-h skeleton was 16.4 ± 0.08 h after the start of the light period, and under the 10-h skeleton, 19.5 ± 0.18 h after the start of the light period. Subtracting the light-period lengths, this means that the 4- and 10-h main light periods place the peaks at hours 12.4 and 9.5, respectively, of the corresponding dark periods. The slope calculated from the 12 values of this experiment is -0.484 ± 0.032 . A similar calculation using values from two successive experiments carried out on the single channel version of the apparatus gave an estimated slope of -0.440 ± 0.075 . Neither of these slopes differ significantly from those derived from earlier data on CO_2 output and on flowering⁶, but, like those, they do differ significantly ($P = 0.01$) from both 0 and –1.

The light effects reported earlier are probably exerted entirely through phytochrome^{4,5}. Perhaps the most striking single phytochrome phenomenon is that of the 'red, far-red switch'. That is, on a nitrate medium, under a basic schedule of one 0.25-h light exposure every 24 h, cultures previously entrained to far-red respond to red by a near-doubling of CO_2 output in 6 h. Conversely, cultures previously entrained to red respond to far-red with a sharp drop in CO_2 output, even though the steady-state pattern elicited by daily far-red would give a peak shortly after the light exposure⁵. The experiment presented in Fig. 2, which may be compared to Fig. 4 in ref. 5, indicates that precisely the same effects are observed in O_2 uptake.

The fact that O_2 uptake by *L. perpusilla* is essentially parallel to CO_2 output in all conditions tested means that the use of this system to investigate photoperiodic timing^{5,6} must proceed through an analysis of respiratory pathways rather than non-respiratory decarboxylations. Beyond this rather specialised point, however, these data also lead to the conclusion that a substantial proportion of the respiration of at least one higher plant may be affected by aspects of the light regime unrelated to photosynthesis.

The applicability of this conclusion to plants in general is suggested by several considerations. Leopold and Guernsey³ observed short-term phytochrome effects on O_2 uptake in

tissues of several species. Few similar studies (for example refs 8–11) have appeared since, but there is abundant, if somewhat contradictory, recent evidence for phytochrome-mediated changes in the levels of various respiratory intermediates^{12–15}. The nightly pattern of CO₂ output—presumably respiratory—by *Chenopodium polyspermum* changes within a few days of a change in the length of the light period¹⁶. In addition, phytochrome clearly mediates light effects on rhythms in plant respiration, respiratory enzymes and intermediates¹⁷. Miyata and Yamamoto¹⁸ suggested that rhythms in *Lemna gibba* respiration involve variations in the activity of the pentose phosphate pathway, while in *Chenopodium rubrum*, Deitzer *et al.*¹⁹ found distinct rhythms in enzymes of that pathway on the one hand and of the tricarboxylic acid cycle on the other. Thus, modifications of the relative activities of these two metabolic systems might underlie the present results.

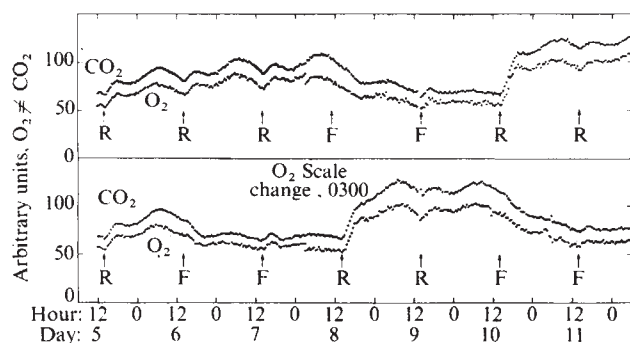


Fig. 2 Rates of O₂ uptake and CO₂ output by cultures on nitrate as sole N source and entrained originally to 0.25 h red light every 24 h, then given red (R) or far-red (F) as indicated. Light sources as in ref. 5. Other details as in Fig. 1. Gaps in the data represent recorder malfunction.

The fraction of total respiration under the control studied here has been estimated^{5,6} at from 30% for daily entrainment patterns (Fig. 1) to 50% in a 'red, far-red switch' (Fig. 2). These may be minimal estimates. Unpublished data indicate that respiration in most of the cultures used is soon limited by crowding, which probably explains the apparent decrease in the proportion of control with time (Fig. 1 of ref. 5). While the parallelism between O₂ uptake and CO₂ output is extremely close in these data, it may not be perfect. The ratio of the arbitrary values, or apparent respiratory quotient, changes slightly in response to experimental variables. Further work will be required to establish that these observations do not result merely from differing gas solubilities or other non-biological factors.

Finally, although the term photorespiration has become virtually synonymous with effects of bright light on the consumption of current or very recent photosynthate, a comprehensive view of metabolic regulation should no longer ignore the fact that overall respiratory rate can be massively, and on occasion rapidly, modified through other light actions. Since these actions may be exerted at times remote from those of actual illumination, and at low energies, the retention of quotation marks in the phrase (and concept) of 'dark' respiration² seems particularly wise. The fact that the respiratory rhythm in *Lemna gibba* seems to be correlated with changes in azide sensitivity¹⁸ is of particular interest since inhibitor-resistant electron transport may be wasteful with respect to phosphorylation²⁰. Thus, in the context of current concern with metabolic efficiency and plant productivity^{1,2}, this work directs renewed attention to light action on phytochrome and to its timing—the photo-periodic aspects of the daily light regime. And although the effects of blue light on *Lemna* respiration are probably mediated entirely by phytochrome⁴, the situation in general could prove

to be even more complex, in view of recent evidence for physiological photoreception by flavin-cytochrome b couples in both fungi²¹ and higher plants²².

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Viral contamination of bovine foetal serum and cell cultures

THE presence of adventitious viruses in cell cultures is well recognised¹, and when the cultures are of primate origin there are serious hazards for the production of human viral vaccines². This is one reason for the increasing use of bovine cell cultures. These cultures, however, are not free from viral contamination³. We found that calf kidney (CK) and calf testis (CT) cells were often infected by non-cytopathic mucosal disease virus (MDV): the cells seemed morphologically healthy but nearly all showed fluorescence with MDV antiserum and rabbit-anti-bovine conjugate. We report here that both the cells and foetal calf serum, an essential growth factor of cell culture medium, are sources of the virus.

MDV (also known as bovine viral diarrhoea virus), a member of the family Togaviridae, is antigenically related to hog cholera virus⁴ and morphologically similar to rubella virus⁵. The role of FCS, as a source of contamination by MDV, was examined by growing CT cells in medium supplemented with either unheated or heated FCS. Heated FCS had been held at 56 °C for 30 min because these conditions inactivated MDV. The incidence of infection of each cell batch grown in unheated FCS was compared with the incidence of infection of the same cell batch grown in heated FCS (Table 1). Fourteen batches of CT cells grown in five batches of FCS were examined. All of the 14 batches of cells grown in the presence of unheated FCS became infected by MDV. In contrast, when grown in the presence of heated FCS, only one of the 14 batches became infected by the virus.

Each batch of CT cells was screened for infection by MDV at several different passage levels (Table 2). Viral infection was not usually detected until passage 6 when 70% of cell batches grown in unheated FCS showed MDV fluorescence. Only 25% of cell batches showed evidence of MDV infection at passage 3.

Table 1 Batches of bovine cell cultures showing MDV fluorescence and grown in the presence of unheated or heated FCS

Cell	FCS	No. positive	No. tested
CT	Unheated	14	14
	Heated	1	14
CK	Heated	10	63
MDBK*	Unheated	1	1†
BT22†	Unheated	1	1‡

The indirect fluorescent antibody test was carried out using antiserum raised in a gnotobiotic calf against the NADL (National Animal Diseases Laboratory) strain of MDV, and a rabbit-antibovine serum conjugated with fluorescein (Nordic Immunology). Pre-inoculation serum was used as a control of fluorescence. CT and CK cell cultures were derived from 1–15 d-old calves, freshly slaughtered each week; trypsinised tissues were seeded into 20-oz bottles and cultured in Eagle's basal salts medium supplemented with 7.5% commercial FCS. Each batch of CT cells was subcultured for up to 15 passages, and CK cell cultures were subcultured until the secondary passage.

*Madin Darby bovine kidney.

†Bovine turbinate.

‡Continuous cell lines; therefore only one 'batch' could be examined.

The other possible source of non-cytopathic MDV, the cells themselves, was examined by fluorescent antibody staining of CK cells which were grown in the presence of heated FCS and subcultured only once. Ten of 63 cell batches examined showed MDV fluorescence. Two continuous cell lines, MDBK and BT22, were also found to be infected by non-cytopathic MDV (Table 1).

Specificity of the fluorescent antibody test was confirmed by neutralisation tests. The agent isolated from CT cells was able to infect uncontaminated CK cells. But when the agent was first treated with antisera to MDV (either a Weybridge reference serum or an antiserum raised at Compton by hyperimmunisation of a gnotobiotic calf with the NADL-MDV strain), CK cells did not become infected. Thus antisera to MDV neutralised the agent isolated from CT cells; no neutralisation occurred with pre-immune control serum.

The results of fluorescent antibody staining were also supported by tests for interference, which were carried out by a plaque technique. A standard inoculum of cytopathic MDV was titrated on Petri dishes of CT cells, which fluorescent antibody staining had shown to be infected by non-cytopathic MDV. The number of plaques produced by the cytopathic virus was compared with the number produced by the same viral inoculum titrated on known, uncontaminated CT cells (Table 3). No more than a twofold reduction in plaque number on the contaminated cells was found.

Table 2 CT cell cultures showing MDV fluorescence at various passage levels

FCS*	Results of FA†	Cell passage no.					
		1	3	6	9	12	15
Normal	No. positive	0	3	10	8	7	6
	No. tested	6	12	14	12	8	6
Heated	No. positive	1	0	1	1	1	0
	No. tested	11	12	14	12	8	6

*Half of each batch of CT cells was grown on medium supplemented with unheated FCS and half on medium supplemented with heated FCS.

†Indirect fluorescent antibody staining as in Table 1.

The conventional interference test, used for screening FCS and bovine cells, relies on detection of a visible inhibition of virus-induced cytopathic effect. This method is obviously not as sensitive as a quantitative plaque technique and probably would not detect the relatively low level of interference as noted in Table 3.

The results indicate two different sources of contamination by non-cytopathic MDV. First, MDV may be found in commercially produced FCS because heat treatment of serum reduces the incidence of infection of CT cells from 100% to 10%. Second, MDV may be indigenous to bovine cells; approximately 20% of batches of CK cells grown in

Table 3 Comparison of number of plaques produced by cytopathic MDV on uninfected CT cells with number of plaques produced on CT cells infected by non-cytopathic MDV

Cell batch/ passage no.	Mean no. plaques per plate $\pm$ s.e.		Student's <i>t</i> test
	Uninfected	Infected	
60/9	48 $\pm$ 8	24 $\pm$ 7	5.131
60/13	21 $\pm$ 7	15 $\pm$ 2	1.774*
63/6†	54 $\pm$ 12	26 $\pm$ 3	4.704
	29 $\pm$ 8	17 $\pm$ 4	2.998
	30–5	18–5	3.485

Standard inocula of cytopathic MDV (NADL) were titrated on Petri dishes of CT cells either contaminated or uncontaminated by non-cytopathic MDV. Mean numbers of plaques produced on five replicates of contaminated CT cells were compared with mean numbers of plaques produced on the same cells but which were uncontaminated. Student's *t* test, $P < 0.05$, 4d.f.

*All comparisons except this one showed significant differences.

†Three different inocula of cytopathic MDV.

heated FCS for only two passages, and on serum shown to be free of MDV by culture of CT cells, are infected by MDV. CT cells grown in normal FCS have to be subcultured several times before showing evidence of MDV infection, and so the viral inoculum in FCS is low.

In the above experiments, CT cell cultures were exposed to five different batches of FCS obtained from one commercial supplier: all batches contained MDV. These results indicate a much higher incidence of contamination than reported previously^{6,7}, and illustrate the inadequacy of commercial screening methods. This inadequacy may be explained by the relative insensitivity of the interference test, often the only method used to detect non-cytopathic MDV, and by the fact that the amount of virus in FCS is probably small.

The presence of MDV in FCS is explained by the ability of the virus to infect the bovine foetus *in utero*⁸. A main supplier of FCS is Australia where infection by MDV is widespread: in 1964 it was shown that approximately 60% (374/625) serum samples taken from cattle throughout the country had neutralising antibodies to MDV⁹. The incidence of foetal infection is unknown, but because each batch of FCS represents a pool of serum taken from about 500 foetuses (for an average batch of 170 l), contamination of a batch requires only a 0.2% incidence of foetal infection by MDV.

Infection of bovine cell cultures by non-cytopathic MDV has particular significance for the production of viral vaccines. Tamoglia¹⁰ tested live infectious bovine rhinotracheitis vaccines from all licensed producers in the United States and found that 8% were contaminated by MDV. Vaccination with such vaccines may give rise to abortions since transplacental infection by MDV can cause death and malformations of the foetus¹¹.

The importance of contamination by MDV with regard to human viral vaccines is unknown, but measles virus

vaccine¹² and a potential respiratory syncytial virus vaccine¹³ are produced on bovine kidney cells grown in the presence of unheated commercial FCS. Contamination of viral stocks also has important implications for experimental virology. For example, in a trial designed to assess the clinical responses of calves to infection by respiratory syncytial (RS) virus¹⁴, the RS viral inoculum, grown in CK cells, was found to contain MDV. If MDV had not been detected, the clinical reactions of inoculated animals would have been attributed solely to RS virus.

Regular screening of bovine cell cultures is essential if viral stocks are to be kept free from MDV. We suggest that the fluorescent antibody test, using a monospecific antiserum, is the most reliable method available for detecting non-cytopathic MDV in cell cultures. Moreover, if FCS is heated before use, the incidence of contamination of cell cultures by MDV will be greatly reduced.

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Neonataly tolerant mice fail to react against virus-infected tolerated cells

In mice, immune effector functions mediated by thymus-derived lymphocytes (T cells) are restricted by the major histocompatibility (*H-2*) gene complex (see ref. 1 for review). Thus, virus-stimulated cytotoxic T cells are specific for both viral antigen(s) and self-cell-surface structures coded in the *K* or *D* region of *H-2* (refs 2, 3). Two general hypotheses have been proposed to explain *H-2* restriction²⁻³. First, T cells possess two independently, clonally expressed, but linked receptors, one for the foreign antigenic determinant and one for the relevant self-marker (dual receptor model); second, T cells have a single clonally distributed receptor for a complex of foreign antigen plus self or for virally altered self-markers (altered self model). To date no conclusive proof exists for one hypothesis or the other. The altered self model is the simpler concept of the two; on the other hand, the fact that cytolytic interactions against minor histocompatibility antigens are *H-2* restricted and that the idiotypic determinants of T cell-receptors are identical with those of immunoglobulins of the same specificity⁶⁻⁸ are probably more compatible with a dual receptor.

In both models, but particularly in the dual receptor model, defining the relationship between tolerance against alloantigens and the capacity to recognise the same alloantigens as self or alternatively as altered self seems to be crucial for understanding of the mechanisms of T cell recognition. Experiments with *H-2^k/H-2^d* radiation chimaeras, that were reconstituted with one parent's haemopoietic stem cells revealed that separate groups of T cells were specific for virus plus *H-2^k* or virus plus *H-2^d* (refs 9-11). The absence of alloreactivity (presence of tolerance) appeared to parallel presence of self-recognition (as proposed in the dual receptor model), or alternatively the presence of recognition of altered self. The present experiments were designed to evaluate whether tolerance to alloantigens and reactivity to tolerated self plus virus parallel each other when tolerance was maintained with minimal chimerism as in the classical Billingham-Brent-Medawar¹² neonatally tolerant mouse model. The results revealed no such correlation.

Neonatal A.AL (*H-2K^k*) mice were injected into the orbital vein with spleen and bone marrow cells of (A.AL × A.TL)*F*₁ (*H-2K^k/H-2K^s*) origin. (W. Streilein and J. Klein, in preparation). Eight weeks later, survivors were grafted with A.TL skin. Mice that did not reject the skin grafts were considered as tolerant (*H-2K^k* tolerant to *H-2K^s*). Tolerant and conventional A.TL (*H-2K^s*), SJL (*H-2K^k*) and B10.BR (*H-2K^k*) mice were then infected with vaccinia virus, and their spleen cells were assayed for virus-specific cytotoxic activity on vaccinia virus infected and uninfected syngeneic or allogeneic target cells 6 d later (Table 1). The degree of chimaerism of the tolerant mice was determined by lysing of their lymphocytes in the cytotoxic test with antisera against *H-2K* haplotypes. Chimerism in the tolerant mice was below 5% since specific anti *H-2K^k* (K-603) antiserum + C' killed >95% of lymphocytes from conventional A.TL mice, lysed less than 5% cells from tolerant mice^{13,14}.

Although vaccinia virus immune spleen cells from tolerant mice lysed infected *H-2K^k* L cells to a high degree they failed to lyse any of the three infected *H-2K^s* targets. The *H-2K^s* targets were lysed very efficiently by A.TL or SJL immune lymphocytes. Therefore, the efficiency with which virus immune spleen cells from allogeneic or tolerant mice lysed the *H-2K^s* targets must be at least 50 times lower than that for immune cells from A.TL or SJL.

This result could suggest the presence of some suppressor mechanism in the tolerant mice's immune spleen cells that prevent expression of virus-specific cytotoxic activity against the infected targets of the tolerated *H-2* type. Yet the cytolytic activity of virus specific T cells specific for *K^s* was not diminished significantly when they were mixed with excess numbers of cells from mice tolerant to *K^s*; any effects observed were comparable to interference caused by unrelated *K^d* spleen cells from B10.D2 mice that were not tolerant to *K^s* (Table 2). Thus suppression seems to be unlikely at least at the level of the effector cell. This experiment, however, cannot exclude the possibility that suppressive mechanisms act during induction of the cytotoxic lymphocytes.

The difference of the results obtained in mice that were rendered tolerant neonatally and the *F*₁ radiation chimaeras may reflect different mechanisms of tolerance induction and/or maintenance. Since these mechanisms are poorly understood this argument remains open. A possible difference may be presence or absence of suppression. Alternatively, the data may suggest that the generation of self plus virus-specific cytolytic T cells is, besides being virus-dose dependent, also dependent on the dose of the relevant self-markers. In P (*H-2^k*) → *F*₁ (*H-2^k/H-2^d*) radiation chimaeras, when the syngeneic and the tolerated allogeneic haplotypes are both expressed in great amounts, virus-specific cytolytic activity is generated against virus in association with both

Table 1 Vaccinia virus specific cytotoxic T cell activity of neonatally tolerant mice

Immune spleen cells (mouse strain, <i>H-2K</i> allele)	Spleen to target cell ratio	L929		% ⁵¹ Cr release from target cells*		SJL		A.SW	
		<i>(H-2K^k)</i>		fibroblasts <i>(H-2K^s)</i>		macrophages <i>(H-2K^s)</i>		macrophages <i>(H-2K^s)</i>	
		Vaccinia	Normal	Vaccinia	Normal	Vaccinia	Normal	Vaccinia	Normal
A.AL tolerant to A.TL (<i>H-2K^k</i> tolerant to <i>H-2K^s</i>)§	30:1	78.4†	22.7	20.9†	15.3	38.0†	40.5	39.9†	34.7
	10:1	92.4†	24.8	21.8†	—	39.4†	—	40.7†	37.0
	3:1	65.4†	20.2	21.2†	—	39.6†	—	42.0†	35.2
A.TL (<i>H-2K^s</i>)	30:1	16.6	24.2	76.6†	18.1	88.0†	42.1	70.4†	35.2
	10:1	17.9	23.2	48.1†	—	73.1†	—	57.0†	36.2
	3:1	15.9	—	37.5†	—	56.1†	—	51.9†	—
SJL (<i>H-2K^s</i>)	30:1	16.9	—	—	—	83.2	41.0	—	—
	10:1	19.9	—	—	—	85.0	—	—	—
	3:1	—	—	—	—	72.4	—	—	—
B10.BR (<i>H-2K^k</i>)	30:1	74.2†	20.1	19.8	—	43.1	41.7	43.3	—
	10:1	66.1†	—	18.5	—	42.6	—	—	—
	3:1	45.7†	—	—	—	41.7	—	—	—
Normal A.TL (<i>H-2K^s</i>)	30:1	12.2	—	—	—	40.2	40.3	41.9	32.2
	10:1	13.6	—	—	—	42.7	—	—	34.2
Normal B10.BR (<i>H-2K^k</i>)	30:1	14.2	20.7	—	—	—	—	—	—
	10:1	—	—	—	—	—	—	—	—
Medium		12.3	19.5	14.0	18.2	40.3	41.0	42.0	33.0

Mice were infected with about 10^7 plaque forming units (PFU) of vaccinia virus (WR strain). The mice were killed 6 d later and spleen cells assayed for cytotoxicity at the spleen cell to target cell ratios indicated. Since only the constitution of the *K* region of the *H-2* complex is relevant to this experiment, the constitution of the other regions is not shown. Where no values are shown the values were not determined.

*L929 continuous line fibroblast cells, SJL secondary fibroblasts and SJL or A.SW peritoneal macrophages were labeled with ⁵¹Cr, washed and infected with vaccinia virus at a multiplicity of about three for 3 h at 37 °C. The % of ⁵¹Cr released was determined after 6 h incubation at 37 °C. The standard errors did not exceed 2.2 for all targets tested.

†Significantly greater than controls ($P < 0.01$).

‡Not significantly different from normal cell controls or medium.

§The *H-2* type of lymphocytes was determined by a microcytotoxicity test^{13,14}.

	anti- <i>K^s</i> + C' (K-119)	% specific lysis anti- <i>K^k</i> + C' (K-603)	C' alone
A.TL	> 95	> 95	> 5
Tolerant	< 5	> 95	< 5

The antisera were K-603 : (C3H.OH × 129/J)_F₁ anti-C3H/HeJ, and K-119 : (A × C57BL/10Sn)_F₁ anti-B10.S.

Table 2 Absence of demonstrable blocking or suppressing activity on virus-specific cytotoxicity in spleen cells from neonatally tolerant mice

Immune spleen cells (mouse strain, <i>H-2K</i> , <i>D</i> allele)	Spleen to target cell ratio	% ⁵¹ Cr release from vaccinia virus infected target cells					
		SJL macrophages (<i>H-2K^sD^s</i>)		L929	J774		
		Numbers of blocking cells added	Admixed blocking immune spleen cells	(H-2K ^k D ^k)	(H-2K ^d D ^d)		
						Tolerant	B10.D2
A.SW (<i>H-2K^sD^s</i>)	30:1	None	78.9	—	—	25.3	30.0
	10:1		84.4	—	—	21.2	30.5
	3:1		65.7	—	—	18.7	—
B10.D2 (<i>H-2K^dD^d</i>)	30:1		52.0	—	—	24.2	92.7
	10:1		50.7†	—	—	22.0	80.4
	3:1		—	—	—	20.7	56.2
A.AL tolerant to A.TL	30:1		56.1†	—	—	90.7‡	73.6‡
(<i>H-2K^kD^d</i> tolerant to <i>H-2K^s</i>)	10:1		58.1†	—	—	87.4‡	57.3‡
	3:1		55.1†	—	—	54.1‡	39.5
A.TL (<i>H-2K^sD^d</i>)	30:1		79.8‡	—	—	22.0	81.2
	10:1	1 × §	89.1‡	81.6*	89.4	22.3	57.1
		2 ×	—	84.7*	76.7	23.7	39.0
	3:1	1 ×	70.1‡	70.9*	69.6		
		2 ×	—	67.8*	68.9		
		9 ×	—	61.1*	60.5		
Normal A.TL			55.6	—	—	23.0	34.7
Medium			54.2	—	—	25.1	33.0

Mice were infected and cytotoxicity tests performed as for Table 1. S.e.m. were below 2.5 except for the blocking data where they were below 3.2.

*None of these values was significantly smaller than those obtained with B10.D2 as blocking cells.

†Not significantly greater than controls.

‡Significantly greater than controls, $P < 0.01$.

§Vaccinia immune spleen cells from the B10.D2 or tolerant mice tested were added to the A.TL immune spleen cells in equal amounts (1 ×) or 2, 3 and 9 times excess amounts.

The *H-2* type of the lymphocytes was determined by microcytotoxicity test^{13,14}.

	anti- <i>K^s</i> + C' (K-119)	% specific lysis anti- <i>K^k</i> + C' (K-603)	C' alone
A	< 5	> 95	< 5
A.TL	> 95	< 5	< 5
Tolerant	< 5	> 95	< 5

haplotypes. In the neonatally tolerant mice studied here, the chimaerism is minimal, sufficient to maintain tolerance, but not to an extent where during infection appreciable amounts of cells would express virus plus alloantigen to trigger measurable quantities of cytotoxicity against these specificities.

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Reassembled HVJ (Sendai virus) envelopes containing non-toxic mutant proteins of diphtheria toxin show toxicity to mouse L cell

PREVIOUS studies from this laboratory have described the preparation, from HVJ virions solubilised with a non-ionic detergent, of a reassembled envelope fraction free of nucleocapsids. Such reconstituted envelopes still retain neuraminidase, haemagglutinating, cell fusion and haemolytic activities^{1–3}. Since intact virions release their nucleoproteins into the cytoplasm after fusion with mammalian cells, it seemed possible that any given macromolecule contained within such a reassembled viral envelope might be introduced into susceptible mammalian cells by this means. We report here the successful application of this method to the introduction of CRM45, a non-toxic mutant protein related to diphtheria toxin⁴, into mouse L cells, a strain that is normally toxin resistant. Although CRM45 is a potent NAD : elongation factor 2-ADP ribosyl (NAD : EF2-ADPR) transferase^{5,6} and thus blocks protein synthesis in eukaryotic cell extracts, it is non-toxic for animal cells because it lacks a C-terminal sequence of amino acids necessary to bind to susceptible cell membranes and thus cannot reach the cell

interior⁷. If a few molecules of CRM45 could be introduced into the living cell cytoplasm, they should block protein synthesis and kill the cells. This has now been accomplished by fusion of CRM45-containing reassembled HVJ envelopes with cultured mouse L cells.

HVJ virions were collected by centrifugation, resuspended in buffer containing CRM45 and divided into two portions. Non-ionic detergent Nonidet P40 (NP40) was added to one portion to solubilise the virions; it was not added to the other which served as a control. After ultracentrifugation of the solubilised virions to remove nucleocapsids and other insoluble material, the envelopes were reassembled by dialysis against phosphate buffered saline (PBS) to remove the detergent and then applied to a Sephadex G200 column to separate reassembled envelopes or native virions from free CRM45 protein. The reassembled envelope fraction was eluted from the gel with the void volume as a single sharp peak (Fig. 1). NAD : EF2-ADPR transferase activity and haemagglutinating activity were both associated with this

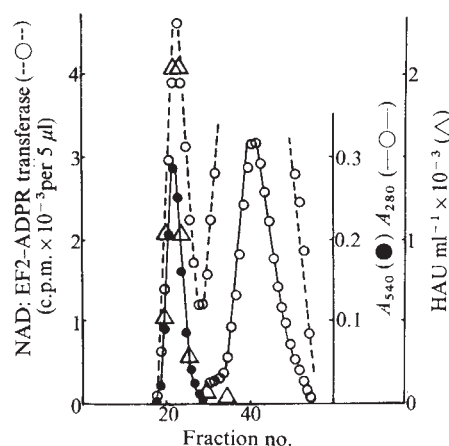


Fig. 1 Separation of reassembled CRM45-containing envelopes from free CRM45. Purified HVJ virions (about 7×10^4 HAU) were collected and resuspended in 1 ml 0.005 M Tris buffer at pH 7.4 containing 5 mg ml⁻¹ CRM45 and divided into two equal portions. To the first was added NP40 at a final concentration of 0.25%; the other served as a control. After 15 min at room temperature, the solubilised virions were centrifuged at 40,000 r.p.m. for 60 min to remove nucleocapsids and other insoluble material. The resulting supernate was dialysed at 4 °C in a Neflex tube against PBS with the buffer changed each day for 3 d. The reassembled dialysed envelope preparation was then chromatographed through Sephadex G200. Ten drop fractions (0.73 ml) were collected and the absorbance of each fraction was measured at 280 nm and at 540 nm. The HVJ virions to which no NP40 had been added were suspended in 0.5% CRM45 solution and dialysed against PBS and chromatographed through Sephadex G200 in the same manner. NAD:EF2-ADPR transferase activity of each fraction was assayed using 2 µl, 5 µl and 10 µl samples by a slight modification of the method of Gill and Pappenheimer⁸. Haemagglutinating activity, determined using chicken erythrocytes, was expressed as HAU ml⁻¹.

peak. A peak of free CRM45 was found in fraction 41 and thus was well separated from the reconstituted envelopes. The CRM45-containing reassembled envelopes were collected and resolubilised with NP40. Resolubilisation resulted in a 3.2-fold increase in enzymatic activity (Table 1). These observations suggest that about 70% of the CRM45 was inside the reassembled envelopes and about 30% was tightly bound to the membrane with its enzymatically active fragment A moiety^{8,9} exposed to the outside. About 2% of the total added CRM45 was recovered with the envelope fraction. The control HVJ virions, suspended in CRM45 at the same concentration, were also filtered through Sephadex G200. The native virion fraction emerged with the void volume as expected, but contained very little ADP ribosylating activity. There was no further increase in enzymatic

Table 1 Enzymic activity of CRM45-containing reassembled envelopes before and after treatment with NP40

Pooled fractions	A_{640}	HAU ml ⁻¹	NAD:EF2-ADPR transferase	
			before NP40 c.p.m. per 5 µl	after NP40 c.p.m. per 5 µl
Reassembled envelopes containing CRM45	0.225	1,500	4,115	12,963*
Intact virions, pre-incubated with CRM 45	1.5	16,000	187	150

*This activity increased about twofold as did that of free CRM45 after brief controlled treatment with trypsin (see ref. 4).

Fractions 21–24 of Fig. 1, both from gel filtration of CRM45-containing reassembled envelopes and from the intact HVJ virions, were pooled. The pooled volume was 2.9 ml in each case. Both suspensions were then solubilised with NP40. Assay for NAD:EF2-ADPR transferase activity was carried out on 2 µl and 5 µl samples after threefold dilution, both before and after treatment with NP40 as described in the legend to Fig. 1.

activity after solubilisation of the virions with detergent (Table 1). Thus CRM45 neither enters the intact virions nor binds non-specifically to their surface.

The enzymatic activity per ml of CRM45-containing envelope suspension was equivalent to about 20 µg ml⁻¹ of diphtheria toxin. The toxicity of the suspension was tested using mouse L cells known to be at least 10,000 times as resistant to intact toxin as HeLa cells. Table 2 shows that 70% and 92% of L cells were killed after short exposure to the CRM45-containing HVJ envelope suspension diluted so as to contain respectively only 10 and 100 haemagglutinating units (HAU) per ml. The same amount (HAU) of either reassembled virion envelopes, or of ultraviolet-killed virus, whether added alone or mixed with free CRM45, showed no toxicity at all for L cells when tested under the same conditions. It is clear that CRM45-containing reassembled HVJ envelopes are toxic for a mammalian cell line. Further experiments suggest that the method is likely to prove of general use for introducing macromolecules into the cytoplasm of living mammalian cells. We experienced no difficulty in introducing ¹²⁵I-labelled IgG into mammalian cell

Table 2 Toxicity of CRM45-containing reassembled HVJ envelopes for mouse L cells

Sample	Number of surviving colonies per dish	
	100 HAU ml ⁻¹	10 HAU ml ⁻¹
Reassembled CRM45-containing envelopes	12	44
Intact HVJ virions plus CRM45 (1 µg ml ⁻¹)	132	129
Reassembled empty envelopes plus CRM45 (1 µg ml ⁻¹)	132	129
Reassembled envelopes	142	155

Two hundred L cells suspended in 2 ml of MEM medium containing 10% calf serum were added to 30 mm plastic Petri dishes and incubated for 7.5 h at 37 °C. The cells were then washed once with 2 ml chilled buffered salt solution (BSS, see ref. 15) before 0.5 ml of the chilled sample was added. After 20 min at 4 °C they were brought to 37 °C and incubated a further 20 min. Two ml culture medium was added and incubation continued for 3 h at 37 °C. The medium was then exchanged for fresh medium and the cells were cultured for 7 d. The cells were finally fixed with methanol and stained with Giemsa. Colony counts were averages of duplicate plates. Control dishes contained 130 and 132 colonies. To prepare samples for test, the pooled fractions of reassembled HVJ envelopes of Table 1 were diluted in BSS 15 and 150 times to final concentrations of 100 and 10 HAU ml⁻¹ respectively. Both ultraviolet-killed HVJ virions and their envelopes reassembled in the absence of CRM45 were diluted to 100 and 10 HAU ml⁻¹. CRM45 was added to the diluted controls at a final concentration of 1 µg ml⁻¹.

lines (L cell, Ehrlich ascites tumour cell and KB cell). The yield of IgG trapped within the reconstituted viral envelopes was about the same as for CRM45.

There are many reports of introduction of substances into living mammalian cells, the most frequently used techniques being the liposome^{10–12} and erythrocyte ghost–HVJ methods^{13,14}. As the procedures for reassembling the envelopes are mild without any drastic mechanical steps and the reassembled HVJ envelopes have specific binding sites to susceptible cells, we believe that the method we have described is also effective for introducing proteins into the cytoplasm of living mammalian cells.

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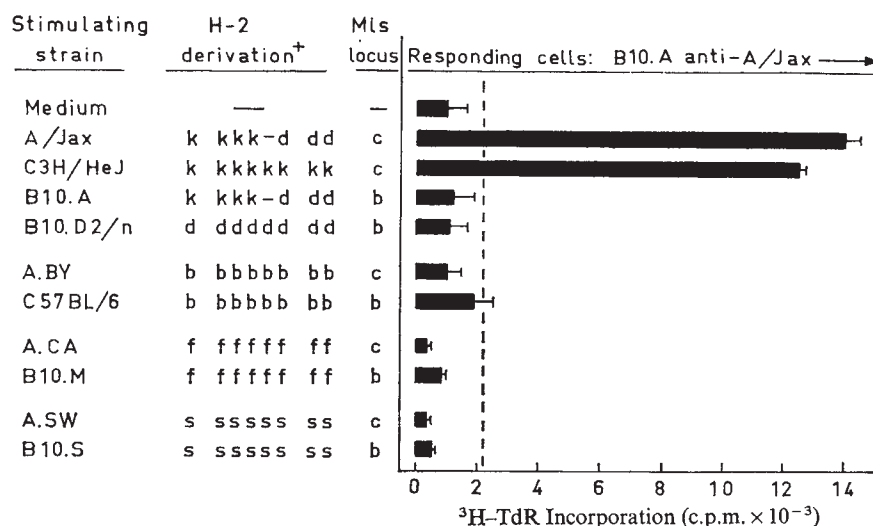
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T lymphocyte responses to *Mls* locus antigens involve recognition of *H-2 I* region gene products

THE major histocompatibility complex (MHC) in the ninth linkage group of the mouse encodes antigens that stimulate strong alloreactions¹. *In vitro* studies have focused on two such reactions, both mediated by T lymphocytes. Cytotoxic T cells can recognise products of the MHC *H-2K* and *D* regions (KD antigens), and can be distinguished by their Ly antigen phenotype from the non-cytotoxic T lymphocytes that give their strong proliferative responses in mixed leucocyte culture (ref. 2) (MLC). These latter cells respond primarily to *H-2 I* region gene products^{1,2}. The products of a second genetic locus in the mouse also stimulate strong MLC responses. This *Mls* locus³ behaves as a single autosomal dominant gene mapping in linkage group 13. It has been defined by studying MLC reactions between MHC identical strains of mice. Interestingly, it has not been possible to induce cytotoxic effector T cells against *Mls* locus antigens⁴. Recent interest in T cell responses to antigen has focused on the crucial role of MHC gene products in antigen recognition (reviewed in refs 5–8). These studies have shown that T cell responses to most conventional antigens involve recognition of both the foreign antigen and self MHC antigens. In parallel with allogeneic reactions, T cells cytotoxic to antigen-modified self cells see that antigen plus self KD molecules, while helper T cells, T cells mediating delayed type hypersensitivity and T cells proliferating *in vitro* to soluble antigens recognise the antigen in association with I region gene products. Thus,



⁺ Denotes K, I-A, I-B, I-J, I-E, I-C, S and D.

Thymidine incorporation in harvested cells was determined by liquid scintillation counting. Data are expressed as mean c.p.m. of triplicate culture wells. Standard deviations are shown. Vertical dashed line is 2 standard deviations above the mean of incorporation in medium stimulated controls.

virtually all T cell responses involve recognition of MHC gene products, with the possible exception of responses to *Mls* locus antigens. Reactions to *Mls* locus antigens, however, are normally tested between MHC identical strains of mice, so responses to *Mls* locus antigens may also involve recognition of self MHC antigens, probably encoded in the I region⁷. Alternatively, the *Mls* locus might represent I region gene duplications translocated to another chromosome^{3,9}. The present experiments were designed to test these ideas. We report that T cell responses to *Mls* locus antigens involve recognition of the products of two gene clusters. One gene cluster is clearly self MHC genes, probably within the I region, while the second is almost certainly the genes of the *Mls* locus. Thus, virtually all T cell responses to non-MHC antigens involve specific recognition of self-MHC antigens in association with another antigenic determinant.

We have isolated T cell blasts from primary MLC reactions between H-2 identical, *Mls* locus differing strains, and measured their secondary *in vitro* proliferative response to fresh stimulating cells from strains sharing either MHC antigens, *Mls* locus antigens, or both with the original

stimulating strain¹⁰. This technique facilitates study of reactions to *Mls* locus antigens in the presence of MHC antigen differences, since the selected blast cells lack reactivity to MHC antigens, at least during the time in which they are studied (48 h).

Two such experiments are presented. The first (Fig. 1) involved blast cells isolated from the reaction B10.A anti-A, and tested with a variety of strains congenic with A but differing in the MHC. It is clear that secondary reactions to *Mls* locus antigens require that the stimulating cell share MHC region genes with the original stimulating strain. Sharing of the rest of the genetic background is not sufficient to give a response. Since C3H/HeJ cells induced reactivation, sharing MHC genes to the left of the I-C sub-region seems to be sufficient. Sharing of *Mls* locus genes with the original stimulating strain is apparently also required for this response, but not sufficient.

A second experiment was designed to determine which portion of the MHC was involved in secondary T cell responses to *Mls* locus antigens (Fig. 2). In this instance, purified T cell blasts from the reaction of B10.BR to

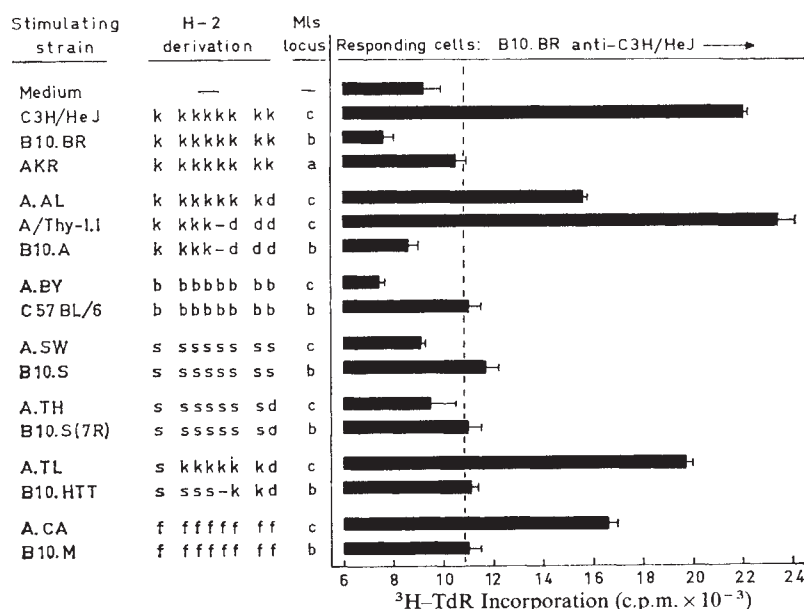


Fig. 2 H-2 I region recognition in secondary MLC responses of B10.BR T lymphocytes primed *in vitro* with X-irradiated C3H/HeJ spleen cells towards a panel of test cells carrying various genetic differences. Primary and secondary MLC carried out as for Fig. 1.

C3H/HeJ were used. C3H/HeJ and all A congenic mice share an *Mls* locus antigen (*Mls*^c), allowing us to test the A congenic mice for secondary stimulatory activity. B10 congenic strains were used as *Mls* negative controls for any reaction to MHC differences. The results confirm the requirement for sharing MRC region genes with the original stimulating strain. Furthermore, the reactions against A.TL and A/Thy-1.1 cells demonstrate that genes mapping between K and I-C are sufficient for stimulation to occur. These experiments do not rule out the possibility of other MHC genes also being sufficient to give stimulation in this system, but both experiments demonstrate an absolute requirement for sharing of MHC genes, since the congenic mice differ only at the MHC region.

These data strongly support the notion that T cell recognition of non-MHC antigens always involves recognition of self MHC gene products. Other non-MHC cell surface antigens have been shown to behave in a similar manner. The male antigen¹¹ and non-H-2 transplantation antigens¹² both induce killer T cells which recognise both the antigen and KD determinants. The *Mls* locus antigens are unique, however, in being the only non-H-2 antigens which induce a strong primary proliferative, non-cytotoxic T cell response; our data suggests they do so in association with I region gene products. Antigens with the characteristics of *Mls* antigens all map to a single locus, whereas there are a wide variety of non-H-2 loci encoding antigens that give rise to cytotoxic effector cells^{11,12}, so this uniqueness may, in part, account for the apparently high frequency of *Mls* reactive cells.

These experiments render very unlikely the hypothesis that *Mls* locus products resemble I region gene products, since *Mls* locus antigens cannot, by themselves, induce T cell proliferation, but must be associated with an I region gene product to do so. Other differences between I region gene products and *Mls* locus antigens emerge from studies of T-B cell collaboration, where I region identity is required for effective cooperation¹³ while *Mls* locus identity is not¹⁴.

Finally, we believe that this system can be applied to the mapping of I region genes. A possible illustration of this is the finding that B10.A anti-A cells do not respond to A.CA, whereas B10.BR anti-C3H cells do respond to A.CA. This reaction pattern does not correspond to any known I region antigen, and thus may point to a new I region determinant. Further studies will be aimed at defining these unexpected cross-reactions.

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Density-dependent phosphorylation of a specific protein in cultured cells

THE phosphorylation-dephosphorylation reactions that regulate the biological activities of many proteins are sensitive to changes in physiological conditions¹. Hormones regulate the phosphorylation of both membrane-bound² and cytosolic proteins^{3,4}, while the phosphorylation of histone⁵ and non-histone chromosomal proteins⁶ changes during different phases of the cell cycle. The cellular density of mammalian cells grown in culture can affect biological properties such as cell growth⁷, locomotion⁸, transport⁹ and enzyme activity^{10,11}. We have examined the phosphorylation of endogenous proteins in cultured mammalian cells at different cellular densities as a means of determining whether cell-cell interactions might affect protein phosphorylation. We report here that the phosphorylability of a specific protein present in several diverse cell lines exhibits density-dependence.

Cell lines designated Balb 3T3 and SV3T3 originated from the Balb A₃₁ and SVT₂ lines, respectively, established by George Todaro (NIH); T3 schwannoma originated from RN₂ lines established by S. E. Pfeiffer (University of Connecticut); AFH, an amelanotic variant of B16 melanoma lines, was obtained from J. J. Sauk Jr (University of Minnesota); the SF human diploid fibroblasts were secondary cultures derived in our laboratory.

AFH melanoma cells, T3 schwannoma and SV3T3 fibroblasts do not exhibit density-dependent inhibition of growth, and grow to high densities (> 10⁷ cells per 100-mm plate). These cell lines were plated at both low and high densities and extracts were prepared 24-48 h after plating. The extracts were incubated with γ -³²P-ATP, and the ³²P-labelled proteins were separated by sodium dodecyl sulphate (SDS) polyacrylamide slab gel electrophoresis. A protein of molecular weight 110,000 (determined by comparison with molecular weight standards) was heavily phosphorylated in extracts from cells which had been plated at a high cell density, but was only slightly phosphorylated in extracts from low density cells (Fig. 1a-c). The *in vitro* phosphorylation of no other protein consistently changed as a function of increased cell density in the cell survey described here. One possible explanation for the increased phosphorylability of this protein in extracts from high density cells could be an increased concentration of this substrate protein. But when cell extracts are incubated with γ -³²P-ATP, only sites available for phosphorylation (that is, which have not been phosphorylated in the intact cell) incorporate ³²P. Thus, another explanation could be an altered regulation of the phosphorylation of the protein *in vivo*.

In addition to comparing the phosphorylation of extracts from cells which had been plated initially at high and low densities, we have also examined extracts from cells which were plated at low density and then grown to high density. Once again, the phosphorylation of this single protein was higher in the extracts from high density cells than from the low density cells (Fig. 1d). We conclude that the phosphorylability of this protein increases when the density of cells on a plate is high, and does not depend on the experimental manipulations used to achieve high cellular density. We have called this protein density-dependent phosphoprotein (DDP).

The effect of cellular density on DDP phosphorylation was also examined in two normal cell lines which exhibit density-dependent growth inhibition. SF, a line of human diploid fibroblasts which grows to a high density (10⁷ cells per 100-mm plate) but then becomes quiescent, also showed a greater extent of DDP phosphorylation at high density than at low density (Fig. 1e). Balb 3T3 mouse fibroblasts normally grow to a low saturation density of about 10⁶ cells per 100-mm plate. These 3T3 cells showed minimal DDP phosphorylation in extracts from both confluent cultures and sparsely populated cultures (Fig. 1f). Serum-stimulated (40 % serum) 3T3 cells,

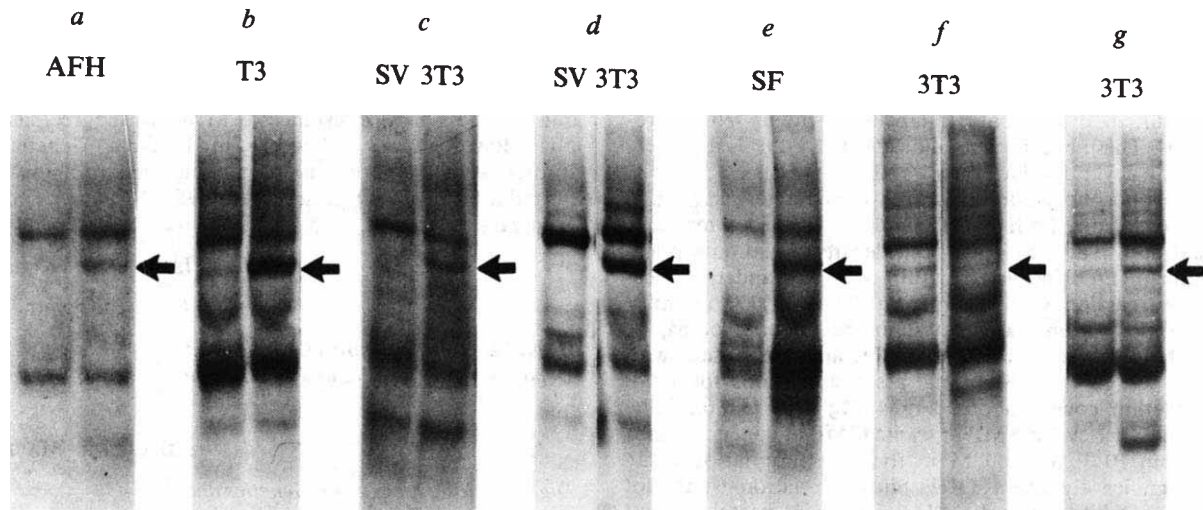


Fig. 1 Autoradiograph of ^{32}P -labelled proteins from mammalian cell lines at low and high density. Various mammalian cell types (a–c, e) were plated at either low density (10^4 – 10^5 cells per 100-mm plate) or high density (10^6 – 10^7 cells per plate) and grown for 24–48 h. The culture media for cells were: DMEM and 10% foetal calf serum for murine SV3T3, Balb 3T3 and human diploid fibroblasts; DMEM and 10% calf serum for rat T3 schwannoma; and RPMI 1640, 10% foetal calf serum and 10% tryptone for murine AFH melanoma. SV3T3 cells (d) were plated on 100-mm plates at low density (10^5 cells per plate) and grown to high density (10^6 – 10^7 cells per plate). Extracts from low density cells were prepared 8 h after plating and those from high density cells 72 h after plating. To obtain sufficient protein from sparse cultures, Balb 3T3 cells were grown in roller bottles. Extracts were prepared from low density cells (before cell contact) 4 d after seeding 100 ml of medium with 2×10^6 cells; high density cell extracts were prepared 8 d after seeding (f). In other experiments, Balb 3T3 cells were first grown to confluence (10^8 cells per plate) on 100-mm plates; medium was then removed and replaced with DMEM and 40% foetal calf serum, and growth continued for 48 h until a high density (4×10^6 cells per plate) was reached. Extracts were prepared from low density cells and from these high density cells (g). In all experiments, cells were grown at 37°C and exposed to a humidified atmosphere of 5% CO_2 . Samples were prepared by rinsing cells with 0.32 M sucrose, and disrupting the cells by sonication. Protein concentrations were determined by the method of Lowry *et al.*¹². The crude sonicates were assayed for endogenous phosphorylation as described by Malkinson *et al.*¹³. The 100- μl reaction mixture contained 70–90 μg of protein, 50 mM (*N*-morpholino) ethanesulphonic acid (MES) pH 6.5, 10 mM MgCl_2 , and 1.0–1.5 μM γ - ^{32}P -ATP (5–20 Ci mmol $^{-1}$). After incubation for 3 min at 30°C the reaction was terminated with an SDS-dithiothreitol 'stop solution', and the tubes were boiled for 2 min. ^{32}P -labelled proteins were separated by SDS-polyacrylamide slab gel electrophoresis¹⁴ and autoradiographs of the dried gels were prepared. Each cell line is represented by a pair of autoradiographs. In each pair, the left autoradiograph was obtained from extracts of low density cells and the right autoradiograph from extracts of high density cells. The arrow indicates the position of DDP on each autoradiograph.

however, grew to densities of 4×10^6 cells per 100-mm plate, and more DDP phosphorylation occurred using extracts from these high density cells than with extracts from sparse plates (Fig. 1g). These experiments with growth-regulated human and mouse fibroblasts indicate that increased DDP phosphorylability also occurs in normal cells after growth to a sufficiently high cellular density.

The following experiment illustrates the reversibility of this density-dependent phosphorylation. SV3T3 cells were plated at a low density (10^5 cells per 100-mm plate). At various times during growth, the plates were scored for cell contact, the number of cells per plate was determined, and cell extracts containing equal amounts of protein were phosphorylated (Fig. 2). DDP phosphorylation was minimal when cells were sparse (before cell contact). When 100% of the cells had established cell contact (10^6 cells per plate), DDP phosphorylation began to increase. When the highest density was reached (10^7 cells per plate), DDP phosphorylation was 8–15 times higher than in sparsely populated plates. When these high density cells were diluted and replated at a low density, the absence of cell-cell contacts was associated with a decreased phosphorylability of DDP. When the cell number exceeded 10^6 cells per plate, DDP phosphorylation again increased.

When endogenously phosphorylated proteins from high density AFH or SV3T3 cells were incubated with pronase ($30 \mu\text{g ml}^{-1}$) at 30°C for 15 min before the addition of SDS, DDP and all other phosphorylated bands were absent from the autoradiographs. Neither RNase nor phospholipase C had any effect on the phosphorylated bands. Boiling the homogenates before incubation with γ - ^{32}P -ATP also abolished all bands from the autoradiographs. Incubation of endogenously phosphorylated proteins with a partially purified preparation of liver phosphoprotein phosphatase (supplied by J. S. Bishop)

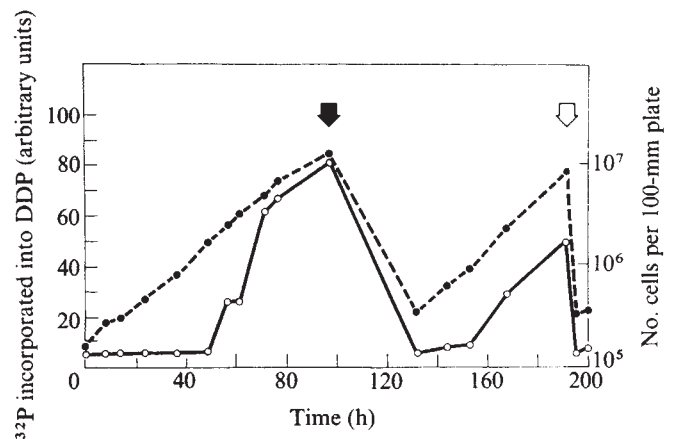


Fig. 2 Amount of DDP phosphorylation in extracts from SV3T3 cells grown to different cell densities. SV3T3 cells were plated at 10^5 cells per 100-mm plate and the medium was changed at 72 h. At the indicated times, cells were removed with 0.05% trypsin and counted. This treatment with trypsin did not affect DDP phosphorylation. Duplicate plates were prepared and assayed for endogenous phosphorylation as described, using 67 μg of protein per assay. When cells had grown to a density of 10^7 cells per plate (indicated by closed arrow), the cells were removed with trypsin, diluted in fresh medium, replated at 10^5 cells per plate and grown again to a high density. At 8×10^6 cells per plate (indicated by open arrow) cells were removed, diluted and replated at 3×10^5 cells per plate. At various intervals the cell number was determined, and extracts were phosphorylated. The amount of ^{32}P incorporated into DDP was determined by scanning the autoradiographs with an Ortec 4310 microdensitometer. The peak height of the optical density is expressed as arbitrary units of ^{32}P incorporated¹⁵. Cell number (●) and DDP phosphorylation (○) are shown.

reduced DDP phosphorylation on the autoradiograph. When a trichloroacetic acid (TCA) precipitate of ^{32}P -labelled proteins from a crude sonicate was incubated at 37°C with dilute alkali, ^{32}P was released within 15 h and no phosphorylated bands were seen on an autoradiograph. Incubation with dilute acid did not result in the loss of ^{32}P -labelled proteins from autoradiographs. These experiments indicate that DDP is a protein containing an acyl phosphate ester bond.

The DDP phosphorylation reaction was characterised (Fig. 3) using extracts from both SV3T3 and AFH melanoma cells which had been plated at a high cell density. Maximum phosphorylation of DDP occurred within 3–5 min, and the phosphorylation reaction was linear from 100 to $1,000\ \mu\text{g ml}^{-1}$ DDP phosphorylation was greatest in the presence of Mg^{2+} , slight in the presence of Mn^{2+} or Co^{2+} , and was absent with either Zn^{2+} or Ca^{2+} . The optimal assay conditions for DDP phosphorylation consisted of $10\ \text{mM Mg}^{2+}$ at pH 6.5. The inclusion of either cyclic AMP or cyclic GMP at concentrations ranging from 10^{-11} to $10^{-4}\ \text{M}$ in the phosphorylation assay did not significantly affect DDP phosphorylation (data not shown). An inhibitor of cyclic AMP-dependent protein kinase enzymes, isolated from rabbit skeletal muscle¹⁶, caused no inhibition of DDP phosphorylation. Thus, in our assay conditions, the kinase catalysing DDP phosphorylation is independent of cyclic nucleotides. Homogenates from high density cells were fractionated by differential centrifugation into soluble and particulate fractions and the endogenous phosphorylation assay was performed with each subcellular fraction. DDP phosphorylation was observed only with the $100,000g$ supernatant, suggesting that neither DDP nor the kinase enzyme which catalyses its phosphorylation are tightly membrane-bound.

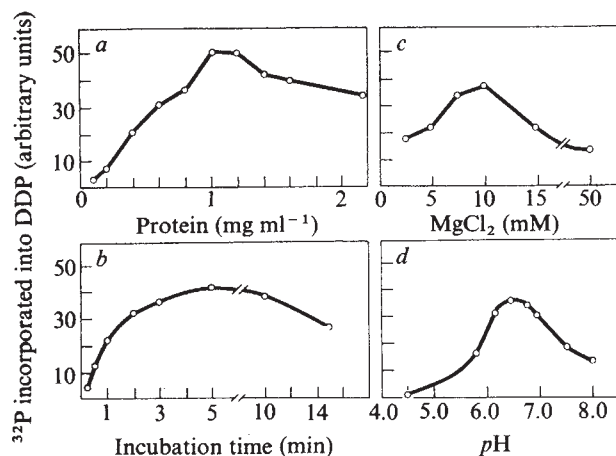


Fig. 3 Amount of DDP phosphorylation in extracts of AFH melanoma (a, c, d) and SV3T3 cells (b) grown to high density, as a function of: a, protein concentration; b, time; c, concentration of MgCl_2 ; d, pH. Standard reaction conditions described for Fig. 1 were used except for the changes indicated. Buffers for the pH curve, at $50\ \text{mM}$, were: pH 4.5, sodium acetate; pH 5.8, 6.2, 6.5, 6.8, MES; pH 7.0, 7.5, 8.0, HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulphonic acid).

Differences in phosphate metabolism have been found between 3T3 and SV3T3 cells, and between high and low density suspensions of 3T3 cells^{9,17}. It is unlikely that increased DDP phosphorylation at high density is related to these changes in phosphate metabolism, for DDP is the only protein whose phosphorylation is consistently affected by density. There are several enzymes¹⁸ and contractile proteins¹⁹ of a monomer molecular weight corresponding to DDP, but it is premature to speculate on the identity of this protein. It is interesting that a phosphorylated protein corresponding in molecular weight to DDP has been observed in foetal rat tissues, but not in adult tissues (A.M.M. and C.E. McSwigan, in preparation). In the cellular slime mould *Dictyostelium discoideum*, the number of freely phosphorylatable sites per cell decreased at the onset

of cell aggregation²⁰. Because many membrane properties change as a function of cellular density²¹, the biological activity of DDP may eventually be found among the contractile proteins or enzymes associated with the plasma membrane.

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Connectivity of the monoamine-containing neurones in central nervous system of leech

THE functional properties of neurones which contain biogenic amines are usually proposed from the results of studies involving ablation or pharmacological intervention¹. Monoamine (MA) neurones are seldom identifiable *in vivo*, which impedes direct investigations of their functions. The central nervous ganglia of leeches are comprised of about 350 neurones, nine of which contain amines as their putative neurotransmitters. These MA neurones are vitally and selectively stained by Neutral Red dye², a procedure potentially facilitating intracellular studies of this population of biochemically related cells. We report here the electrophysiological properties of the red-staining neurones within the central nervous system (CNS) of the leech.

Chains of five to eight segmental ganglia were dissected from horse leeches, *Haemopsis marmorata* (Say), and their MA cells were stained by 0.01% (w/v) Neutral Red in leech saline³ for about 30 min. Nine widely scattered neurones stain within the three anterior-most ganglia, while seven stain within each of the 18 posterior ganglia (Fig. 1). These neurones included the two Retzius cells (RZ, 50–65 μm), two pairs of lateral cells, one pair ventral (VL), the

other dorsal (DL), and a posterior, unpaired medial cell (M). An extra pair of MA cells (E) was seen within each of the three anterior ganglia. These seven small (15–20 μm) intraganglionic neurones shared histochemical properties with the RZ (refs 4, 5), which contain the indolealkylamine, serotonin (5-hydroxytryptamine, 5-HT)⁶. The interconnected 21 segmental ganglia innervate the body by means of paired lateral roots. A stainable neurosoma is situated in each anterior root, and this pair of anterior root cells (AR) probably contains the catecholamine, dopamine². The large RZ send axons to the periphery^{4,7}, while the small MA cells appear to restrict their connections to the CNS.

Stained cells were impaled with glass micropipettes (4 M potassium acetate) with tip resistances of 20–50 M Ω . Membrane potentials and impulse activities were controlled with currents injected through the recording electrodes by means of balanced bridge circuits. Potentials were recorded on tape (FM, HP 3960) and permanent records were usually produced by playback at $\frac{1}{4}$ speed into an oscillographic recorder (HP 7404). Such speed reduction avoids attenuation by the oscillograph of the high frequency components of the action potentials.

Stained RZ cells have resting potentials (40–60 mV) and non-invading action potentials (30–55 mV) indistinguishable from those in unstained preparations². Furthermore, the stained pair of RZ remain coupled by an efficacious electrotonic junction. The small MA cells have small action potentials (usually 5–15 mV) which do not actively invade their somata. We recorded simultaneously from a Retzius cell and each of the small ganglionic MA neurones, and representative results are shown in Figs 2 and 4. The impaled MA neurones usually have tonic impulse activities and post-synaptic potentials, and frequently produce bursts of impulses. These bursts usually occur with slowly rising depolarisations of 5–15 mV. As Fig. 2a shows, the bursts

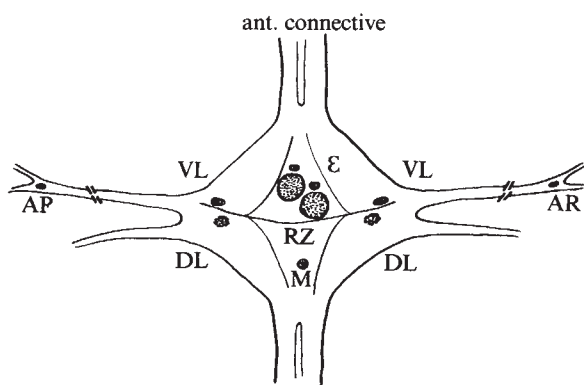


Fig. 1 Diagram of the ventral aspect of a leech segmental ganglion showing the positions of the MA-containing neurones which are stained by Neutral Red dye.

in the RZ and the small cell usually occur in temporal register, suggesting that they receive a common excitatory input⁸. Cross correlograms of the time intervals between the depolarisations of the MA cell pairs support the suggested pattern of a common excitor. We see this pattern of nearly synchronous depolarisations and impulse bursts between RZ and both DL and M (Fig. 2a) as well as VL and E (not shown here). To map more completely the responses of the MA cell population, we impaled and recorded simultaneously from three pairs of small, bilaterally homologous cells—VL–VL, DL–DL and E–E. As Fig. 2b (VL–VL) shows, these cell pairs also undergo nearly synchronous depolarisations which generate impulse bursts. We have recorded from pairs of non-homologous

small cells and found a similar pattern of common excitatory bursts by the following pairs: VL–DL, VL–M, E–M and VL–E (Fig. 2c).

Thus the entire population of MA neurones shares an excitatory input and often fires synchronous bursts of impulses. The addition of 20 mM Mg^{2+} , which blocks chemical synaptic transmission in leeches⁹, abolishes these depolarisations and bursts. In view of this finding, we

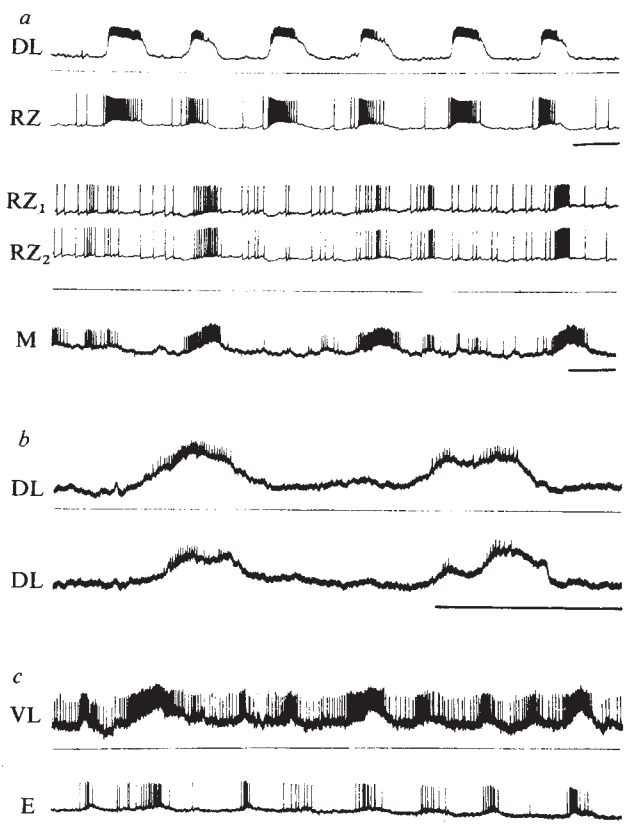


Fig. 2 Intracellular recordings of the spontaneous activity in the red-stained MA neurones of the leech *Haemaphysalis*. Calibrations: 10 mV, 5 s. a, Top panel showing the slowly rising depolarisations and the resulting impulse bursts in DL (top trace) and RZ. The lower panel shows three simultaneous recordings from the pair of RZ and an M cell. The RZ maintain perfect impulse synchrony during burst and interburst periods. M depolarises, generating bursts at similar times to the RZ; however, M impulses do not match those of RZ in a 1 : 1 fashion. b, Slow depolarisations of both DL cells generate impulse bursts. The membranes of both cells show similar slow potential changes, but the action potentials are not 1 : 1. c, Recordings of the depolarisations and action potential bursts in VL (top trace) and E. In this record the impulse frequency of VL is higher than that of E. The magnitudes of the depolarisations appear to differ because of the different gains of each trace.

initiated pharmacological experiments on the chemical synaptic nature of the excitatory depolarisations. Serotonin (10^{-5} M) neither enhances nor induces bursts by MA neurones; 5-HT (the putative transmitter of these cells) hyperpolarises RZ, abolishing all spontaneous impulse activity. Acetylcholine (ACh) is a candidate transmitter for it depolarises and excites RZ into impulse activity¹⁰. Atropine is a competitive inhibitor of ACh at muscarinic receptor sites and it effectively blocks the slow depolarisations and impulse bursts by RZ (Fig. 3). The depolarised bursts return after the atropine is washed from the preparation. Furthermore, the anticholinesterase eserine

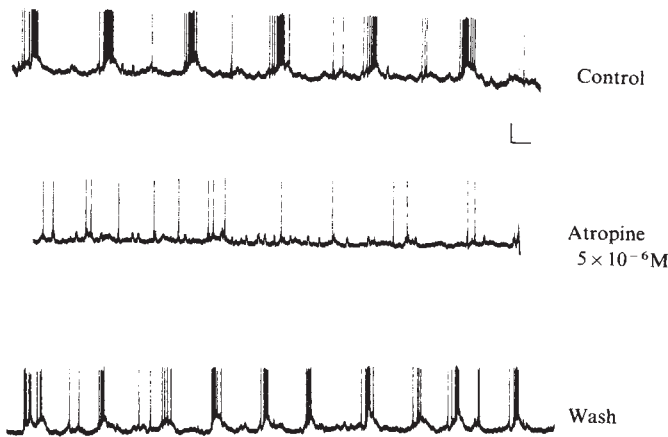


Fig. 3 RZ activity in a stained, bursting preparation. The top panel is a control with depolarisations and bursts at a period of 20–25 s. Atropine blocks the slow depolarisations, but not other unitary excitatory postsynaptic potentials (middle). After washing (bottom), depolarising bursts return but at a period of about 12 s. Calibrations: 10 mV, 5 s.

(5×10^{-4} M) increases both the amplitude and duration of the spontaneous depolarisations seen in the RZ. These eserine-enhanced depolarisations can also be blocked by addition of atropine. We always saw bursts in the small MA cells whenever depolarisations occurred in RZ, and as these depolarisations seem to be mediated by a common excitator, most pharmacological experiments were done with the larger, more accessible RZ. We have verified the effects of atropine on both VL and M cells. Therefore, the central synaptic input which depolarises the MA neurones within the leech ganglion is probably cholinergic.

We passed currents into RZ while monitoring the effects on small MA cells in order to determine the nature of any interconnections among the aminergic population. Inward current directly hyperpolarises RZ and also hyperpolarises the small cells to a lesser degree (Fig. 4a). These d.c. potential shifts persist in a high concentration of Mg^{2+} , and increase monotonically with increasing currents. In one case each nA into an RZ produced a potential change of 0.38 mV in DL. Thus the RZ is coupled to each of these small cells by an ohmic junction: M and E (Fig. 4) and VL and DL (not shown). In addition, hyperpolarisation of each of these small cells will also produce a d.c. hyperpolarisation of the RZ. The RZ are coupled to one another by a low resistance pathway such that an impulse in one cell produces an electrotonic potential in the other which exceeds impulse threshold, and therefore the RZ maintain impulse synchrony (Fig. 2a). The small MA cells are coupled to the RZ by a higher resistance junction. Each RZ impulse can be detected within the small cell somata as an electrotonic potential of about 0.5 mV. These potentials are resistant to Mg^{2+} and have a latency of less than 1 ms. In high Mg^{2+} , a current-induced burst of RZ impulses will electrotonically depolarise a small MA cell to impulse threshold. Experiments on the homologous, small cell pairs show electrotonic coupling between E–E, DL–DL and VL–VL (Fig. 4b). Similarly electrotonic coupling is seen between the following pairs of small, non-homologous cells: VL–DL, VL–M and E–M (Fig. 4c). Hyperpolarisations are usually more effective than are depolarisations for all these MA cell junctions.

Thus all nine monoamine-containing neurones within the leech ganglion seem to be electrotonically intercoupled. Electrotonic interconnections provide a route by which positive feedback can generate bursts of impulses¹¹. Such bursts are produced by the injection of currents into these MA neurones; however, anodal break stimulation (cessation of hyperpolarisation) is more effective in generating a self-sustaining burst than is depolarisation (Fig. 4a and c).

These anodal break bursts can be induced both in preparations showing no spontaneous bursts, and out-of-phase with the timing of the excitator in those showing spontaneous synchronous bursting. Occasionally we can generate an anodal break burst by the MA neurones in high Mg^{2+} saline. Thus the electrotonic coupling among the MA cells is at least in part responsible for burst formation.

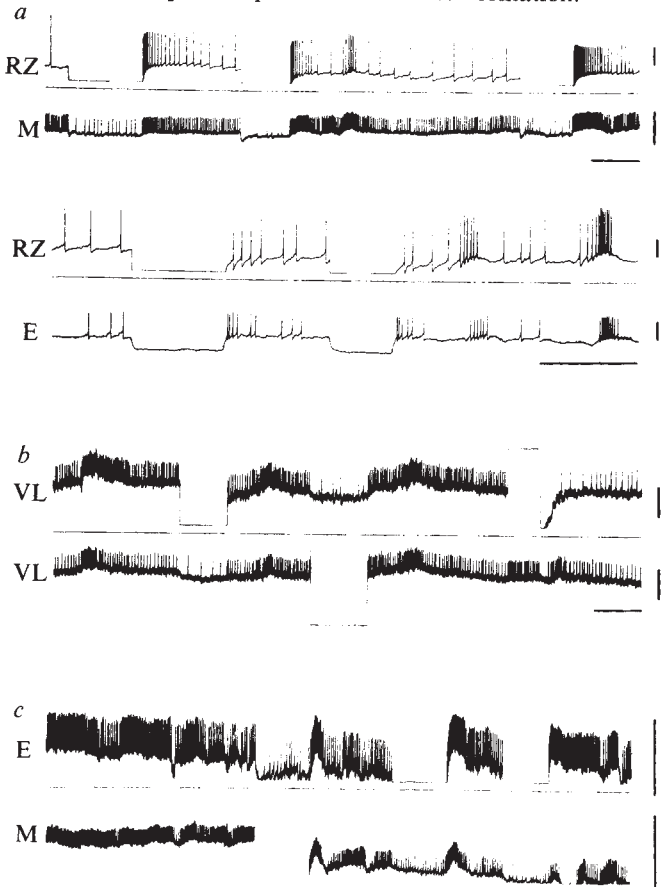


Fig. 4 Electrotonic interactions of the MA neurones in the leech. *a*, In the top panel, a hyperpolarisation of RZ (top trace) produces a d.c. shift in M. These cells have a slight tendency toward anodal break bursts. Note the spontaneous, shared burst in the centre of this record. *b*, Coupling between the paired VL cells. Hyperpolarisation of either cell causes a d.c. shift in the other. Depolarisation of the VL in the top trace slightly depolarises the other increasing its impulse frequency. Note the three spontaneous, shared bursts between the current injections. *c*, Coupling between E and M. Hyperpolarisation of either cell hyperpolarises the other. This preparation had no clear spontaneous bursts; however, cessation of hyperpolarisation generates large, self-sustaining bursts.

The intraganglionic MA cells share a common cholinergic input, and are electrotonically intercoupled. Either of these patterns of connectivity can produce bursts by the MA cells. Presumably the presence of both patterns assures a concerted impulse activity. We do not know why the monoamine-containing neurones burst in concert as their functional role is unknown, except for a peripheral function for RZ in controlling the secretion of mucus from the skin of the leech¹². Thus, the functioning aminergic neurones in the leech are both identifiable and experimentally accessible.

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Post-mortem changes implicate adenine nucleotides and pyridoxal-5'-phosphate in regulation of brain glutamate decarboxylase

GLUTAMATE decarboxylase (GAD) is the enzyme responsible for the synthesis of γ -aminobutyric acid (GABA), an important neurotransmitter in the CNS (ref. 1). Alterations in GAD activity have been implicated in epilepsy, drug-induced convulsions and neurological diseases such as Huntington's chorea and parkinsonism²⁻⁵. Although this enzyme has been under study for over 20 yr, little is known of the molecular mechanisms by which its activity is regulated. Recent results suggest that the degree of saturation of GAD by its cofactor, pyridoxal-5'-phosphate (pyridoxal-P), *in vivo* could be determined by a balance between the rate of dissociation of the cofactor from the enzyme brought about by glutamate and the rate of association of the cofactor with the apoenzyme, which is inhibited strongly by the nucleotides ATP and ADP (ref. 6). Thus, it seemed likely that degree of saturation of GAD by pyridoxal-P *in vivo* might be low and might increase as a consequence of post-mortem changes in the levels of ATP and ADP. Additional activation *in vitro* during preparative steps might also occur because nucleotide levels would be reduced during homogenisation. Data presented here confirm these expectations and implicate the adenine nucleotides and pyridoxal-P in the regulation of GABA synthesis.

The percentage saturation of GAD by pyridoxal-P is defined here as the activity of GAD measured in the absence of added pyridoxal-P as a percentage of the activity measured in the presence of saturating levels of the cofactor (100 μ M). This approach is possible because the

cofactor is tightly bound to the active site in the absence of glutamate and cannot be removed by either dialysis or Sephadex column chromatography^{6,7}. As it has been shown that there is a time-dependent inactivation of the enzyme in the absence of pyridoxal-P and in the presence of high concentrations of glutamate, GAD was incubated for only 5 min with 1 mM glutamate. We estimate that the loss in activity is no more than about 5% in the absence of pyridoxal-P in these conditions. When ATP was added to the homogenate to prevent the association of pyridoxal-P with GAD, we used a concentration of ATP fivefold greater than the concentration of ATP required for maximal inhibition of activation by low levels of pyridoxal-P in order to overcome loss of ATP by hydrolysis⁸.

If brain extracts were prepared in the conventional manner by decapitating the animal, dissecting the brain at room temperature and homogenising in the absence of ATP, we found that the enzyme was 84% saturated by pyridoxal-P (Table 1). If ATP was added to the homogenising medium, however, the degree of saturation fell significantly to 60% ($P < 0.001$). The difference between these values (25% saturation) represents the *in vitro* activation of the enzyme. Similar significant decreases in the percentage saturation were also observed when ATP was included during the homogenisation of brain samples obtained by freeze-blowing⁹ or from heads decapitated into liquid nitrogen (Table 1). To determine whether this *in vitro* activation could be attributed to the binding of endogenous cofactor to the apoenzyme, the concentration of the endogenous pyridoxal-P was directly measured in HClO₄ homogenates using tyrosin apodecarboxylase⁹. The results confirmed that there was sufficient pyridoxal-P to account for this activation (0.5-1.0 μ M). Further evidence of an *in vitro* activation of the enzyme was provided by the observation that the degree of saturation of the enzyme was significantly lower (65%, $P < 0.01$) when 2% rather than 10% homogenates (ratio of tissue weight to total volume) of conventionally dissected brains were prepared (without ATP). Thus, there seems to be a significant increase in the degree of saturation of the enzyme by pyridoxal-P during preparative procedures *in vitro*.

Table 1 Effects of methods of killing and conditions of homogenisation on the percentage saturation of brain GAD

Method	GAD activity (nmol per mg protein per 5 min)				GAD activity (nmol per mg protein per 5 min)			
	No pyridoxal-P	Homogenised without 100 μ M pyridoxal-P	Saturation (%)	n	No pyridoxal-P	Homogenised with 5 mM ATP 100 μ M pyridoxal-P	Saturation (%)	n
a, Decapitated and dissected at room temperature	6.3 $\pm$ 0.2	7.5 $\pm$ 0.2	84 $\pm$ 5	4	5.0 $\pm$ 0.2†	8.3 $\pm$ 0.4	60 $\pm$ 2	4
b, Decapitated into liquid nitrogen	6.1 $\pm$ 0.4	7.9 $\pm$ 0.2	77 $\pm$ 3	4	3.2 $\pm$ 0.4	7.3 $\pm$ 0.5	43 $\pm$ 3	5
c, Freeze-blowing	4.0 $\pm$ 0.3*	6.7 $\pm$ 0.2†	59 $\pm$ 2	3	2.6 $\pm$ 0.1	7.5 $\pm$ 0.3§	35 $\pm$ 2	4

Saturation was determined by dividing activity obtained in the absence of added pyridoxal-P by total activity obtained in the presence of saturating pyridoxal-P (100 μ M). Whole brain from male Sprague-Dawley rats (200-250 g) was removed by three different methods. In (a) rats were decapitated and the brains were dissected out at room temperature. Each was then cut along the midline and half was homogenised in about 9 volumes of 5 mM ATP (pH 6.5); the rest was homogenised in a similar volume of ice-cold deaerated distilled H₂O. Homogenisation was performed within 2 min of decapitation and samples were immediately centrifuged at 16,000g for 30 min at 4 °C. The supernatant was removed and diluted 1:1 with buffer to give 100 mM imidazole-acetate, pH 6.5, and 5 mM mercaptoethanol. Two ml of each diluted supernatant was then passed over a column of Sephadex G-25 (bed volume 70 ml) at a flow rate of approximately 2 ml per min to remove ATP, endogenous glutamate, pyridoxal-P, and other low molecular weight compounds. The column had been pre-equilibrated with buffer at the same concentration and kept in the cold. Five ml of column effluent was collected and used for analysis of GAD activity both in the presence and absence of pyridoxal-P (100 μ M). The procedure used for the enzymatic assay was similar to that previously reported¹⁷ except that the enzyme was incubated for only 5 min and with only 1 mM glutamate. We have previously shown a one-to-one correspondence in the production of CO₂ and GABA from U-¹⁴C-glutamate⁷. In (b) rats were decapitated and the heads were frozen in liquid N₂. After dissection at 0-2 °C each brain was ground to a fine powder and divided into two equal fractions for homogenisation and further treatment as presented in method (a). In (c), brain samples were rapidly removed by freeze-blowing, each was ground to a fine powder and divided into two equal fractions for homogenisation and further treatment as for (a). In all cases samples with saturating pyridoxal-P (100 μ M) were significantly different from paired samples without pyridoxal-P ($P < 0.02$). Comparing corresponding samples analysed in the absence of pyridoxal-P, significantly lower values were obtained in all cases when the samples were homogenised in 5 mM ATP ($P < 0.01$). A similar comparison of samples analysed with saturating pyridoxal-P showed a significant difference only in the freeze-blow method ($P < 0.05$). All values are mean $\pm$ s.e.m.

*Significantly different from similarly treated samples in (a) and (b), ($P < 0.02$).

†Significantly different from similarly treated samples in (a), ($P < 0.05$).

‡Significantly different from similarly treated samples in (a) and (b) ($P < 0.01$).

§Significantly different from similarly treated samples in (a), ($P < 0.05$).

Post-mortem activation of GAD in the intact brain can be seen by comparing the results obtained with ATP-treated homogenates of brains collected by freeze-blowing and the results obtained with similar homogenates of brains dissected at room temperature (Table 1). The freeze-blowing technique removes brain samples and freezes them to liquid nitrogen temperatures within 1 s thereby preventing post-mortem changes in the levels of adenine nucleotides and other energy metabolites⁸. We found that the percentage saturation of the enzyme in homogenates of brain dissected at room temperature (60%) was significantly greater than the percentage saturation in homogenates of freeze-blown brains (34%, $P < 0.001$). The difference between these values (26%) is the post-mortem activation in the intact brain. The activation resulted in an almost twofold increase in the amount of holoenzyme present in the homogenates as indicated by the enzyme activity measured in the absence of pyridoxal-P. Decapitation into liquid nitrogen also reduces the post-mortem activation as shown by the finding that the percentage saturation in ATP-treated homogenates from animals in this group (43%) was significantly lower than the value obtained with similar homogenates from brains dissected at room temperature ($P < 0.001$). A significant post-mortem activation was also observed with samples homogenised in the absence of ATP. In this case, the degree of saturation of the enzyme in brains dissected at room temperature (84%) was significantly greater than the degree of saturation in samples from freeze-blown brains (59%, $P < 0.001$). Since freeze-blowing prevents the rapid post-mortem hydrolysis of ATP which accompanies anoxia⁸, the relatively low degree of saturation in these freeze-blown samples could be attributed to the presence of sufficient endogenous ATP which reduces substantially the amount of binding of pyridoxal-P to the apoenzyme. A similar low degree of saturation was not observed in samples from animals decapitated into liquid nitrogen. It has been shown, however, that marked post-mortem changes in the levels of brain metabolites including a 40% reduction in the brain level of ATP occur during freezing by this method⁸. Thus, there is apparently an insufficient amount of endogenous nucleotides present in these homogenates to prevent the activation of GAD.

Our results demonstrate that brain GAD undergoes both a post-mortem activation by pyridoxal-P in the intact brain and a further activation *in vitro* during preparative procedures. The high degree of saturation (84%) observed in homogenates of brains dissected at room temperature are attributable to these effects. When both types of activation were minimised by homogenising freeze-blown brain samples in 5 mM ATP, the enzyme was only 35% saturated. Clearly, this represents an average for many brain regions and compartments and the actual degree of saturation in any particular compartment may differ significantly from this value. The results do indicate, however, that brain GAD is, in general, only partially saturated with pyridoxal-P. Because of the two types of activation reported here and the glutamate-promoted, time-dependent inactivation of the enzyme^{6,7}, previously reported estimations of the degree of saturation of the enzyme by pyridoxal-P *in vivo* may not reflect the actual values but instead may largely be the result of the experimental conditions. These same considerations also suggest that studies dealing with alterations in the relationship between pyridoxal-P and GAD in drug-induced or pathological states may need to be reconsidered.

It has been demonstrated repeatedly that brain GABA levels increase rapidly after death¹⁰⁻¹³. The post-mortem activation of GAD in the intact brain described here may be a factor contributing to the post-mortem change in GABA levels.

The post-mortem activation of GAD together with the known effects of adenine nucleotides and inorganic phosphate on the interaction between pyridoxal-P and GAD

(refs 6, 14), support the idea that these compounds are involved in the mechanism controlling the rate of GABA synthesis. Although whole brain levels of ATP are quite constant under normal conditions, it is conceivable that fluctuations in ATP levels may occur in various compartments such as presynaptic endings during periods of altered physiological activity¹⁵. Kennedy *et al.*¹⁶ provided strong evidence that changes in the functional state of nervous tissue result in changes in energy metabolism. Such changes might alter the degree of saturation of GAD by pyridoxal-P and thereby mediate changes in the rate of GABA synthesis. On the other hand, the interactions between GAD, pyridoxal-P, and the adenine or other nucleotides may merely set the general level of activity of the enzyme and regulation may be accomplished by other, as yet unknown, factors.

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Effects of dietary choline on nicotinic acetylcholine receptors in brain

THE administration of choline by injection¹ or diet² increases the concentration of acetylcholine (ACh) in the brain. Choline also increases the activity of tyrosine hydroxylase³ (TOH) in dopaminergic neurones of the caudate nucleus that receive cholinergic inputs. This response can be blocked by atropine, indicating that it is due to the post-synaptic action of ACh. The post-synaptic alteration of TOH activity suggests that the level of ACh also regulates the concentration of ACh receptors (AChR). To produce large differences in brain ACh, we have studied two groups of rats, one deprived of choline and the other loaded with choline. We found high concentrations of ACh in the brain associated with increased amounts of nicotinic AChR.

Male, Sprague-Dawley rats, 60 d old (230-300 g) from five litters were assigned to either choline-deficient or choline groups using the split-litter technique. Choline-deficient animals were maintained *ad libitum* on a diet lacking choline (ICN Biochemicals) and were given distilled water to drink. The choline groups were maintained on the same diet but choline chloride 15 mg ml⁻¹ (Sigma) was added to the distilled water in the drink-

ing bottle. All animals were housed in groups of four to a cage. After 21 d, they were decapitated. The brain and hind leg muscles from each animal were processed individually to extract and quantify the receptors present. All procedures were carried out at 4 °C. Each individual tissue was homogenised in ten volumes of 0.32 M sucrose and 10 mM sodium phosphate, pH 7.4. The homogenate was centrifuged at 100,000g for 60 min. The pellet was resuspended (ten volumes of original weight) in 10 mM sodium phosphate, pH 7.4 and 1% Triton X-100 and shaken gently for 105 min. The homogenate was then centrifuged at 100,000g for 60 min. The resulting supernatants (S_2) of brain and muscle were assayed for AChR using ^{125}I - α -bungarotoxin which binds to these receptors with unparalleled affinity and specificity⁴. The nicotinic AChR from brain are similar to the AChR from muscle and electric fish in that they are located at the synapse and have similar toxin binding kinetics, solubility, isoelectric point and hydrodynamic properties⁵⁻¹⁰. Radioactive toxin was prepared by the solid phase lactoperoxidase method yielding a monoiodinated product (specific activity 1.2×10^4 Ci mol⁻¹) and the assay system was based on the use of small columns of CM-50 (Pharmacia) to retain excess ^{125}I -bungarotoxin while allowing receptor-toxin complexes to pass through¹¹. This is the opposite principle to the DEAE filter disk assay¹². All assays were carried out in duplicate and parallel samples were pre-incubated with a 100-

significantly different between the two groups. The daily intake of choline by the choline group was approximately 250 mg per animal. The body weight gain throughout these experiments was not significantly different between the two groups. (See Tables 1 and 2).

Additional rats were maintained in the same conditions and the brains were sectioned into cerebral hemispheres (including olfactory bulbs), remaining forebrain, brain stem and cerebellum, to determine whether there were regional differences in the relationship of AChR to the dietary manipulation. Significant differences in the density of AChR were observed in the cerebral hemispheres (48% increase in the high choline group) and forebrain (43% increase) thus accounting for most of the whole brain differences. A slight but insignificant increase was found in the brain stem. In agreement with other reports, only minimal amounts of AChR were found in the cerebellum¹³⁻¹⁵. Brain weights and extracted protein did not differ between the groups for any brain section.

Thus, these studies suggest that the receptor concentration is positively related to ACh level. Because the range of choline consumption used in the two groups was within the range of variation that could occur in the human diet³, we suggest that alteration in diet can alter brain function by controlling not only the amount of ACh released but also the density of receptors with which it may interact. This observed correlation

Table 1 Effects of choline and choline-deficient diets on brain weight, body weight, whole brain protein and nicotinic AChR concentration in whole brain and muscle

Group	Brain weight (g)	Body weight (g)	Total brain protein mg (S_2 supernatant)	pmol toxin per g brain wet weight	pmol toxin per mg brain protein	pmol toxin per g muscle wet weight	pmol toxin per mg muscle protein
Choline	2.07 ± 0.02	116 ± 6.9	114 ± 2.0	3.29 ± 0.13*	0.056 ± 0.001†	0.3549 ± 0.04	0.0152 ± 0.001
Deficient	2.06 ± 0.02	128 ± 4.7	120 ± 5.1	2.61 ± 0.12	0.042 ± 0.001	0.3359 ± 0.04	0.0138 ± 0.002

Values are expressed as mean ± s.e.m. of 11 animals. Aliquots of supernatant were incubated with ^{125}I - α -bungarotoxin for 60 min at room temperature and then assayed. Radioactivity was determined with a Nucleus γ scintillation counter at an efficiency of 50%. Protein was determined by the method of Lowry *et al.*¹⁸ using bovine serum albumin as a standard.

* $P < 0.01$, Student's t test.

† $P < 0.001$, Student's t test.

fold excess of unlabelled toxin. Specific binding was taken to be the difference between the two assays with and without isotope dilution by unlabelled toxin. There was a 26% increase in the concentration of specifically bound ^{125}I - α -bungarotoxin in the brains of animals which were maintained on the choline diet when computed in proportion to protein extracted ($P < 0.001$) or wet weight ($P < 0.01$). The absolute amount of non-specific toxin binding was less than 25% in all cases and did not differ significantly between groups. No significant differences were observed in the case of AChR from muscle. The increase in brain AChR was not due to malnutrition or a non-specific effect on growth, for brain protein and brain weight were not

between transmitter level and receptor density has significant implications for the relationship between cholinergic neurones and behaviour. Indeed it has been reported that a patient with tardive dyskinesia has been successfully treated with large doses of choline¹⁶. Although little is known about the development and functional implications of AChR in brain, ACh has long been implicated in the physiological basis of several forms of behaviour¹⁷, including memory and learning. We are currently investigating the relationship between diet-induced changes in AChR and learning in the developing and adult rat. The possibility of altering such behaviour by dietary manipulation has profound implications.

Table 2 Effects of choline and choline-deficient diets on brain weight, brain protein and nicotinic acetylcholine receptor concentration in brain sections

Group	Section	Brain weight (g)	Brain protein (mg) (S_2 supernatant)	pmol toxin per g wet weight	pmol toxin per mg brain protein
Choline	Cerebral	1.24 ± 0.05	76 ± 4.8	4.32 ± 0.56	0.067 ± 0.004*
Deficient	hemispheres	1.23 ± 0.04	84 ± 11.6	3.23 ± 0.43	0.045 ± 0.004
Choline	Remaining	0.33 ± 0.03	14.06 ± 1.23	0.3804 ± 0.02*	0.071 ± 0.004†
Deficient	forebrain	0.27 ± 0.04	13.13 ± 2.06	0.2424 ± 0.02	0.050 ± 0.004
Choline	Brain stem	0.20 ± 0.02	6.96 ± 0.88	0.2217 ± 0.02	0.060 ± 0.008
Deficient		0.25 ± 0.04	9.46 ± 2.9	0.2181 ± 0.03	0.055 ± 0.004
Choline	Cerebellum	0.29 ± 0.01	15.34 ± 0.83	0.0339 ± 0.01	0.008 ± 0.002
Deficient		0.30 ± 0.01	14.26 ± 1.3	0.0382 ± 0.01	0.007 ± 0.002

Values are expressed as mean ± s.e.m. of three or four animals.

* $P < 0.01$, Student's t test.

† $P < 0.001$, Student's t test.

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Electrophysiological distinctions between the taste and smell of amino acids in catfish

VERTEBRATES possess two principal systems, olfaction and taste, adapted to respond to peripheral chemical excitation. Olfaction in terrestrial vertebrates is the more acute chemosensory system, having lower thresholds to volatile stimuli than the gustatory thresholds to nonvolatile compounds^{1,2}; thus, olfaction was termed the 'distance' sense and gustation the 'contact' sense. With fishes, however, chemical stimuli are present in the aqueous medium and it is difficult to distinguish between olfactory and gustatory stimuli or to explain the development of two chemical senses in the primitive aquatic vertebrates. Fishes exhibit important morphological distinctions between olfactory and gustatory systems both at the periphery and centrally³, and yet, in catfish, amino acids are the most effective stimuli known for both systems^{4,5}. This sensory overlap does not reflect a simple duplication of receptor function, however, as the olfactory and gustatory systems of catfish differ in amino acid sensitivity, specificity and response characteristics, which could allow for the functional separation of the chemical senses in an aquatic environment.

Channel catfish, *Ictalurus punctatus*, approximately 70–200 g, were immobilised with Flaxedil (gallamine triethiodide; 0.1 mg per 100 g body wt) and positioned in a Plexiglas container with aerated well-water containing MS 222 (tricainemethane sulphate; 1:8,000 dilution) perfusing the gills throughout the experiments; this combination of Flaxedil and MS 222 provided for a more stable preparation than either agent alone. Stimuli dissolved in well-water, approximately pH 7.8, were introduced into a continuous flow of well-water directed to the maxillary barbel or olfactory capsule, and dilutions were determined by photodensitometry of dye solutions. Taste solutions were diluted at least to 60%, and olfactory stimuli at least to 25% of their applied concentrations. For the taste experiments, the eye was removed and a branch of cranial nerve VII (facial) to the maxillary barbel was exposed and placed on a Pt–Ir wire

electrode and covered with mineral oil to prevent drying. To record olfactory receptor responses, a flap of tissue dorsal to the olfactory capsule was removed and a metal-filled glass capillary electrode tip plated with Pt-black⁶ with a ball diameter between 15 and 25 μ m was positioned between adjacent olfactory lamellae near the midline. Both gustatory and olfactory electrical activity were amplified by a conventional capacity-coupled preamplifier, passed through an averaging circuit (Sanborn Model 350–1400 amplifier operated on the a.c. linear mode) with a rise time of 1.0 s and displayed on a pen recorder. The underwater electro-olfactogram (EOG) (ref. 7), an olfactory mucosal slow potential⁸, was recorded with calomel electrodes via Ringer-agar filled capillary pipettes, amplified by a direct-coupled amplifier and displayed on a pen recorder. An essential requirement for successful recording preparations was the use of freshly obtained fish. A rapid decline of olfactory and gustatory receptor sensitivity occurred with increased holding time in the laboratory⁹.

Catfish olfactory and gustatory responses revealed many similarities, but were functionally distinguishable by four criteria: phasic-tonic response characteristics, amino acid thresholds, neural spike synchrony and ordering of effective amino acids. The asynchronous neural response of the maxillary

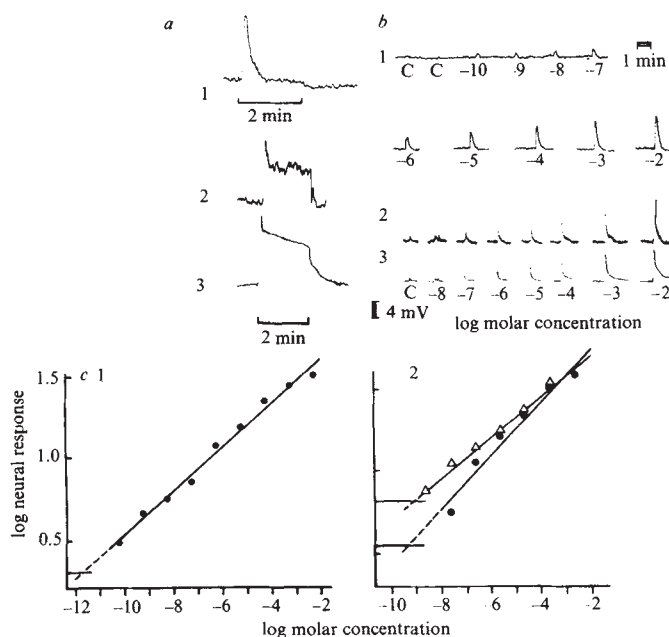


Fig. 1 *a*, Catfish taste and smell responses to a 2-min stimulation with 0.1 mM L-alanine; summated taste recording exhibits only a phasic response (1), while summated olfactory neural (2) and EOG (3) responses show both phasic and tonic components. *b*, Responses to applied concentrations of L-alanine (taste) and L-cysteine (smell); neural taste (1) and simultaneously recorded olfactory neural (2) and EOG (3) responses to applied stimulus concentrations are shown. Lack of olfactory tonic component due to short stimulus duration. *c*, Power relations¹⁹ between chemoreceptor responses and amino acid stimulus concentrations. (1) Taste neural responses; $\log R = 0.132 \log c + 1.84$, $r = 0.99$. (2) Olfactory neural (▲) and EOG (●) responses; $\log NR = 0.125 \log c + 1.95$, $r = 0.99$; $\log EOG = 0.163 \log c + 2.06$, $r = 0.98$. Taste and olfactory neural responses exhibit similar slopes (range 0.07–0.14); EOG slope is somewhat larger (range 0.14–0.29); dotted lines show intersection of linear regression lines with control responses yielding threshold values. Taste is more sensitive than smell in catfish for the compounds tested. All plotted values have been corrected for dilution and are estimates of the stimulus concentrations at the receptors; taste solutions were diluted by 0.2 log unit and olfactory solutions were diluted by 0.6 log unit as determined by photodensitometry of dye solutions. The olfactory neural response to 10^{-2} M L-cys (b2) was omitted from the graph (c2) due to partial synchronisation of the neural spike activity^{11,12}. The processing of this partially synchronous activity by the amplifier averaging circuit resulted in the larger than expected response amplitude, and was therefore not used in determining the olfactory neural threshold.

barbel taste nerve to an amino acid stimulus revealed a quickly adapting phasic response (Fig. 1, a1) with thresholds averaging $10^{-11 \pm 1.7}$ M (Fig. 1, b1, c1) for the more effective amino acids. The absence of the tonic component of the gustatory response was due possibly to adaptation occurring at the synapse between the taste bud cells and the innervating axon¹⁰.

Neural and EOG recordings from olfactory neurons, neuroepithelial cells which do not form synaptic connections until the receptor cell axons reach the central nervous system in the olfactory bulb, exhibited a phasic component followed by a tonic response which remained throughout the stimulus duration (Figs 2, 3). Olfactory thresholds were less than those observed for the taste system and averaged $10^{-8.4 \pm 1.0}$ M (Fig. 1, b2, c2). Also, a partial synchronisation of the axonal spike activity¹¹ occurred with the more responsive preparations when long inter-stimulus intervals (> 5 min) and high concentrations ($\geq 10^{-4}$ M) of the more potent amino acids were utilised. Self stimulation of the olfactory organ by the EOG has been a suggested mechanism for the neural synchrony¹². Gustatory neural activity, however, always remained asynchronous.

The difference in olfactory and gustatory thresholds is not simply a reflection of the differences in the neural recording techniques. The results with the Pt-black ball method (olfactory neural recording), which samples a smaller population of neurons than the Pt-Ir electrode (gustatory recording), are consistent with those obtained from the EOG recordings, which measure the voltage change due to the current flow through the extracellular resistance of the olfactory epithelium from all the receptors being stimulated⁸.

Chemoreceptive specificity of both systems was determined with several amino acids and their derivatives. Both olfactory and gustatory responses were strongly influenced by the position of the amino group. Amino acids having an amino group in the α position (those derived from proteins) were the most effective. Olfactory and gustatory receptors were also able to discriminate enantiomeric pairs, indicating that the chemoreceptors involved possess a corresponding chirality. The L-isomer of an amino acid was always more effective than its D-form, although certain D-amino acids were more stimulatory than other L-forms. A major difference, however, between olfactory and gustatory responses to amino acids in the catfish involved the amino acid side chain. Although both chemosensory systems of catfish were able to detect extremely dilute concentrations of amino acids, each system was specific for particular side groups of the amino acids. The more effective amino acid stimuli based on the neural responses of both chemical systems are listed in Fig. 2; however, neither list correlates with the amino acids essential for growth in this species¹³.

The taste system was highly responsive to the amino acids of low molecular weight having unbranched and uncharged side chains. A major exception was the response to L-arginine, the second most effective taste stimulus, which is a positively charged, higher molecular weight amino acid. The ability of an amino acid to initiate a large gustatory response fell off rapidly after arginine, and the tenth compound in Fig. 2, L-threonine, elicited a response only 50% of that of the most effective chemical, L-alanine, tested at equal stimulus concentrations.

The olfactory system was not as specific as the taste system and was responsive to a group of amino acids. The sulphur-containing amino acids, L-cysteine and L-methionine, were most effective, with less of a decrease in effectiveness as one proceeds down the order in comparison with taste. The tenth compound, L-threonine, produced a response of approximately 75% of that of the most effective olfactory stimulus tested at equal molar concentration. In general, EOG recordings reflected a similar listing of effective amino acid stimuli, but the order was not identical with that for the neural recordings.

Amino acids are likely to be important in various aspects of fish behaviour. Both cartilaginous and bony fishes orient towards an amino acid source¹⁴⁻¹⁶; however, the relative importance of olfaction and taste in the detection of the amino acids stimulus and in the initiation of specific behavioral responses is generally unknown. In catfish, conspecific chemical communication by pheromones is thought to be mediated entirely by the olfactory sense¹⁷, and feeding behaviour primarily by the gustatory sense¹⁸. If certain amino acids or combinations of amino acids provide chemosensory information concerning social or feeding behaviour, then a difference between the olfactory and gustatory receptor responses could code for the appropriate behavioural responses. Although the olfactory and gustatory receptor mechanisms are not understood, each system may have more receptor sites for particular amino acids physiologically relevant to that system.

Whether amino acids such as alanine and arginine, the most effective taste stimuli tested, signal a natural food source in the animal's environment, or whether compounds such as cysteine and methionine, the most efficacious olfactory stimuli tested, are of pheromonal nature, is yet to be determined.

The present experiments indicate that although the olfactory and gustatory systems of the channel catfish are highly sensitive to the α -amino acids, each system responds differently to its chemical environment as determined by electrophysiological recordings.

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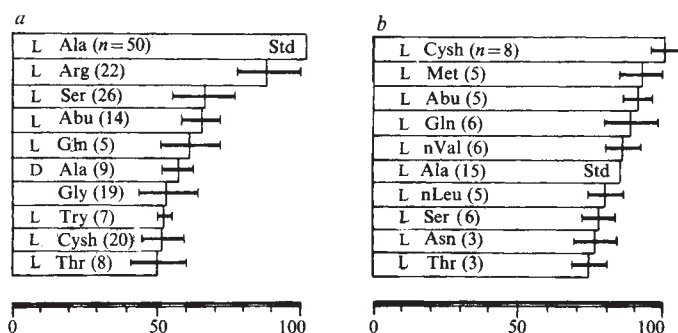


Fig. 2 Comparison of stimulatory effectiveness of the ten most potent amino acids tested on the maxillary barbel and olfactory mucosa at 0.1 mM applied concentrations; estimated concentrations at the receptors were 6×10^{-5} M for the taste (a) and 2.5×10^{-5} M for the olfactory (b) stimuli as determined by photodensitometry of dye solutions. Relative position of each chemical is represented as a percentage of the response to the standard (Std), 0.1 mM L-alanine. n, number of fish tested. Bars indicate s.d.

Calcium utilisation during anaerobiosis induced by osmotic shock in a bivalve mollusc

DURING aerial exposure, some littoral marine bivalve molluscs, for example *Mytilus*¹ and *Mercenaria*^{2,3}, have been shown to close their valves and respire anaerobically. The major by-product of anaerobiosis in the clam *Mercenaria mercenaria* is succinic acid which is neutralised by the dissolution of calcium carbonate previously deposited in the shell^{2,3}. The ability of bivalves to respire anaerobically has been well documented, an early review on this subject⁴ records a variety of species which are able to survive long periods in the absence of oxygen. Most evidence for anaerobic metabolism in bivalves is based on observations that on return to aerobic conditions, oxygen consumption rapidly rises to levels above normal^{5,6}. A similar 'overshoot' in heart rate has been reported^{1,7,8}. These observations have been explained as representing an increase in circulatory and respiratory activity associated with the repayment of an 'oxygen debt' incurred during the period of anaerobiosis. The changeover from aerobic to anaerobic respiration in bivalves has been reported to occur when the oxygen tension of the surrounding water falls to very low levels or to low tide when the bivalves may close the valves^{7,9,10}. We report here results which are part of an extensive study of osmotic and behavioural responses of *Scrobicularia plana* (Da Costa), a euryhaline estuarine species, to varying concentrations of seawater and demonstrate the use of calcium from the shell to buffer the products of anaerobiosis. The concentrations of sodium, potassium, magnesium and calcium were measured by atomic absorption spectrophotometry in the body fluids and medium under different conditions of osmotic stress at 10 °C.

When *Scrobicularia* were transferred from aerated 30‰ seawater to aerated 6‰ seawater, the concentrations of Na⁺ and other ions, except Ca²⁺, in mantle cavity fluid and blood fell gradually to the level of the external medium within 14 d (Fig. 1). At first the valves remained closed for long periods but after 7 d pumping activity took place for

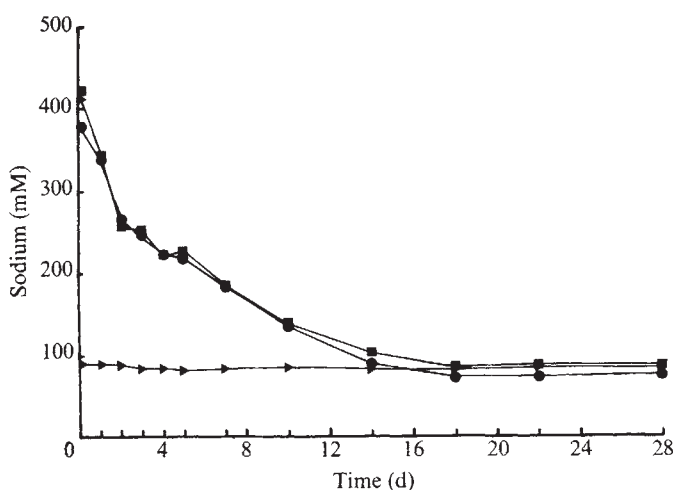


Fig. 1 Na⁺ concentrations in *S. plana* transferred directly from 30‰ seawater (day 0) to 6‰ sea water in well aerated conditions (■), mantle cavity fluid; (●), blood from the ventricle; (▲), medium. Each point is the mean of four determinations made on samples pooled from 12–14 individual *S. plana* selected randomly. Throughout the experiment, the animals were fed regularly on *Tetraselmis* and the medium was changed every 24 h. Ionic determinations throughout were also made on pericardial fluid and pedal haemolymph with similar results which have been omitted for clarity.

more than 50% of the time. In contrast to the other ions, the calcium concentration in the blood rose to a maximum value of about 30 mM (about three times the normal concentration in 30‰ seawater) within 5–7 d of the transfer, after which time the concentrations of calcium in all body fluids dropped towards that of the external medium (Fig. 2a). From day 18, a low stable level of calcium was maintained comparable with previously published values for

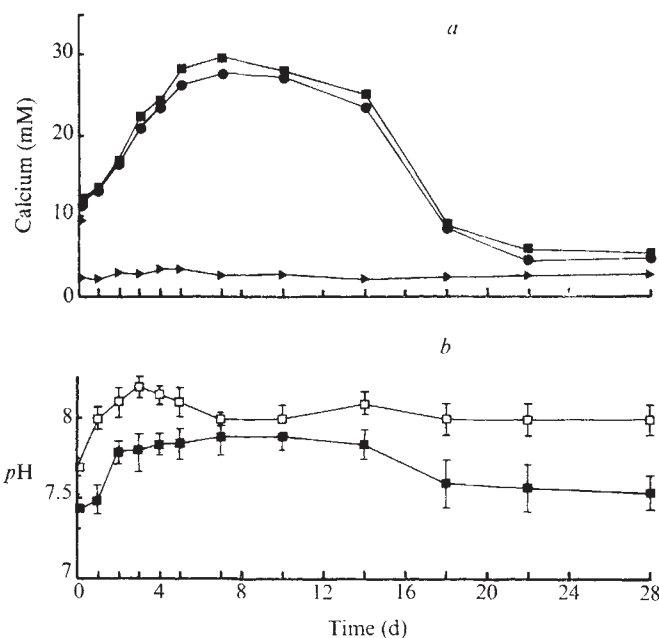


Fig. 2 a, Ca²⁺ concentrations of *S. plana* transferred directly from 30‰ seawater (day 0) to 6‰ seawater. Procedure and symbols as in Fig. 1. b, pH of mantle cavity fluid, medium (symbols as in Fig. 1) and extrapallial fluid (□) from same animals as (a). Extrapallial fluid obtained by drilling the right valve between the adductor muscles. Bars represent s.d. of 10 samples from each of 10 animals. Determinations by Ingold micro pH assembly with Radiometer, M63 pH meter.

other bivalves. Indeed the rise in calcium concentration has not been reported previously because former studies have determined calcium concentrations after a long acclimation period of 3–4 weeks (refs 11–13). The pH of the extra-pallial and mantle cavity fluids changes in a manner similar to calcium (Fig. 2b).

The increase in calcium concentration over the first 7 d is correlated with our observations of the behaviour of *S. plana* under these extreme changes in osmotic concentration. Recordings of valve movements and gill pumping activity show that after reduction of the salinity of the medium, animals largely remain closed but gradually expose the mantle tissue and from the seventh day begin to interact more with the medium, the increase of valve movements and pumping activity coinciding with the fall in calcium concentration and pH. During the initial period when the valves of *Scrobicularia* are closed, we have shown by microdiffusion and thin layer chromatography that ammonia accumulates in the mantle cavity and that succinic acid is the major organic acid accumulated in the tissues. This has been confirmed by De Zwaan (personal communication).

To determine whether the rise in calcium is due to anaerobic metabolism during the acclimation period, *Scrobicularia* were placed in 30‰ oxygen-free seawater under nitrogen. The calcium levels in all body-fluids rose slightly to 14–16 mM and remained at that level over the 6-d period (Fig. 3a, A). During these 6 d the valves remained

open. On the sixth day (Fig. 3a, B), the bivalves were osmotically stressed by changing the medium to 6‰ oxygen-free seawater, resulting in valve closure and inactivity. The concentration of calcium increased reaching a maximum of about 30 mM after which it fell towards that of the medium (Fig. 3a, C). The pH value of the mantle cavity fluid (over the first 6-d period) is lower than that of the medium, as in *Scrobicularia* respiring aerobically in normal seawater (Fig. 3b), but upon transfer to 6‰ oxygen-free seawater, the pH values correspond with those of Fig. 2b. On the 13th day some *S. plana* were closed forcibly by counteracting the hinge ligament with rubber bands and within 3 d the calcium concentrations in the body fluids of these animals increased by 50% (Fig. 3a, D). In *Scrobicularia* kept in 30‰ oxygen-free seawater with their valves forcibly closed, there is a rapid increase in the calcium concentration over a 2-d period to about the same level as those reached by animals in 7 d when subjected to an osmotic shock under aerobic conditions (Fig. 4). After

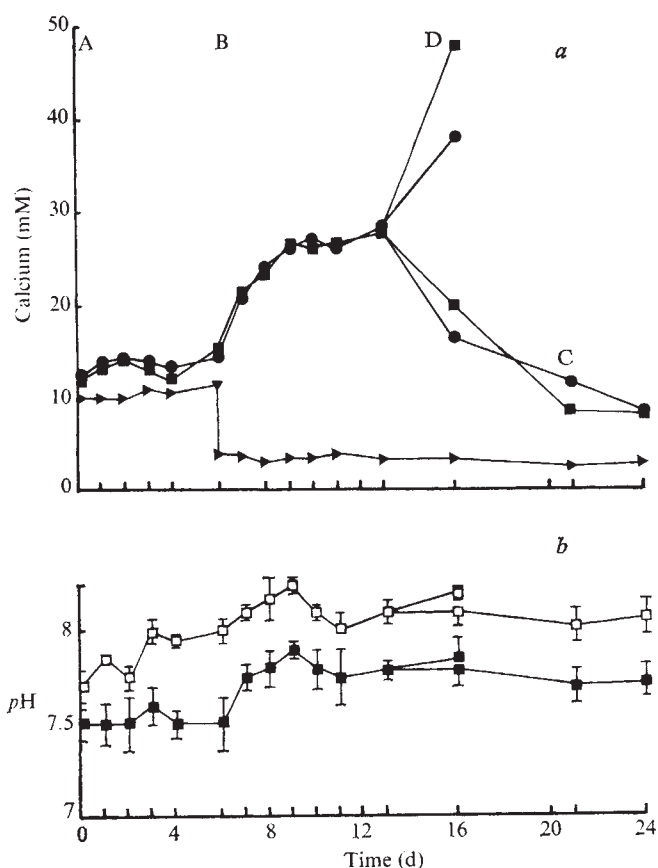


Fig. 3 a, Ca^{2+} concentrations of *S. plana* placed in oxygen-free 30‰ seawater on day 0 (A). (Symbols and procedure as in Fig. 1.) After 6 d the animals were additionally subjected to osmotic stress, by immersion in oxygen-free 6‰ seawater (B and C). After a further 7 d the valves of 20 animals were forcibly closed (D). b, The pH values in animals under the same conditions as Fig. 3a, determined as in Fig. 2.

the second day, the valves were allowed to gape normally and the calcium concentrations dropped to normal levels within 2 d. Unlike animals subjected to osmotic shock, those in oxygen-free normal seawater kept their valves open and continued pumping activity. We suggest that continuous water flow through the mantle cavity may explain the lack of a noticeable rise in calcium concentration, for in these conditions no anaerobic metabolites

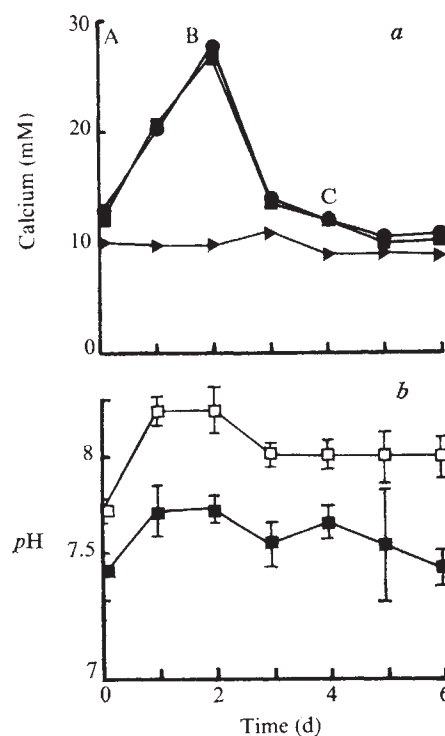


Fig. 4 a, Ca^{2+} concentrations and b, pH values of *S. plana* with valves forcibly closed transferred from aerated 30‰ seawater to oxygen-free seawater (A). After 2 d the bivalves were allowed to gape normally while remaining in the same medium (B). After a further 2 d the medium (30‰ seawater) was aerated (C). Samples and analytical procedure as in Figs 1 and 2.

will accumulate and in consequence no buffering will be required.

Adverse environmental conditions, such as osmotic stress, may cause *S. plana* to close its valves for periods of up to 7 d. During prolonged valve closure respiration is anaerobic and, due to lack of ventilation of the mantle cavity, metabolites, for example succinic acid and ammonia, accumulate. A parallel increase in calcium ion concentration, probably by dissolution of the shell, is thought to act as a means of buffering these metabolites during an initial period of acclimatisation to the extreme salinity change.

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X-ray microanalysis of freeze-dried ultrathin frozen sections of a regulating epithelium from the snail *Otala*

MANY epithelial cells are known to regulate the flow of water and ions across the epithelium. This has been observed in various animals¹ and is probably an essential feature of all living organisms. Hibernating *Otala lactea* (Mollusca, Gastropoda, Pulmonata) can survive long periods of drought without access to water by regulating the evaporative water loss from the mantle epithelium². Machin² has shown that these snails are as efficient as insects at controlling water loss when they are hibernating, yet when they are active and crawling they lose water at the same rate as does a free water surface. To achieve this the animals produce an osmotic gradient across the mantle epithelial cells of approximately 4.5 Osm kg^{-1} from the cell base to the apical plasma membrane². X-ray microanalysis of freeze-dried frozen sections has shown that the levels of silicon, chlorine, potassium, iron and zinc are significantly greater in hibernating (regulating) mantle tissue than in tissues taken from normal actively crawling snails, and a gradient can be observed within these cells.

Hibernating snails were kept in a dry and undisturbed place in the laboratory² while others, which were active, were enclosed in a glass tank with lettuce leaves. Small samples (1 mm^3) of mantle tissue were excised from both hibernating and active snails within 20 s of their removal from their containers. The tissue was frozen on to brass specimen holders⁵ by immersion into liquid nitrogen slush at -210°C (ref. 6). Blocks of tissue were carefully trimmed so that the square blockface included a vertical section through the superficial layers of the mantle epithelium. Trimming and sectioning (130 nm) were done in a cryostat containing an ultramicrotome^{5,6} at temperatures at which ribbons of ultrathin sections could just be obtained, that is control blocks at -72°C ; regulating at -76°C . 'Dry' ribbons of sections were pressed into contact with Formvar-coated nickel grids⁶⁻⁷, freeze dried at their cutting temperatures for 3 h in the cryostat and in the presence of dry nitrogen, warmed slowly to -15°C overnight and brought to ambient temperature in the presence of dry nitrogen. They were then coated with 20–30 nm of carbon to prevent rehydration⁷.

Sections were analysed either in EMMA 4 (analytical electron microscope) equipped with LINK system energy dispersive detector or in the AEICORA analytical microscope (courtesy of Kratos Ltd, AEI Scientific Apparatus). The specimens were held in graphite inserts to reduce the background readings. Analytical conditions were carefully controlled and matched between both instruments—accelerating voltage, 60 kV; beam current as measured by Faraday cage, 4.0 mA; spot size, 100 nm; overall c.p.s., 200–700; counting time 100 s.

Gupta *et al.*³ have studied the distribution of three ions within an insect Malpighian tubule by analysing hydrated 'thick' frozen sections of $1\text{--}2 \mu\text{m}$. In our experience there are considerable advantages in the use of freeze-dried ultrathin frozen sections. The morphology of the cells is sufficiently clear to relate the structures seen in ultrathin frozen sections to those seen in sections of conventionally fixed and embedded material. The good resolution in our micrographs is due to the initial thinness of the section and the removal of water by freeze drying. This also means that there is less lateral spread of electrons around the area of the probe and consequently leads to an improvement in analytical resolution with good peak to background ratios.

Analysis of tissue from ribbons of ultrathin sections of consistent thickness makes it possible to express the results by volume (that is $P-b$) rather than by mass (personal communication from H. Höhling). We felt that the results expressed in this way have greater biological significance than relative mass fractions ($((P-b)/W)$, where $P-b$ is the integrated peak minus background for an element, and W is the continuum or measurement of mass), provided that sections are of uniform thickness and freeze drying causes no lateral redistribution of labile elements. The continuum

measurements for the regulating and control cells showed that there was no significant difference in section thickness (means, where $n =$ at least 30 measurements, regulating $3,382 \pm 94$; controls $3,387 \pm 231$). Previous work suggests that there is no significant redistribution of diffusible elements during sectioning or freeze drying, and Gupta *et al.*³ have claimed that there were no 'parallel changes in either the characteristic counts or the distribution of elements' in their sections before and after drying.

The subcellular detail of the regulating epithelium is shown in the electron micrographs (Fig. 1*a, b*, and *c*)⁹. Figure 1*a* shows an ultrathin frozen section and Fig. 1*b* an enlargement of the apical region. The results of the X-ray microanalysis of this and other

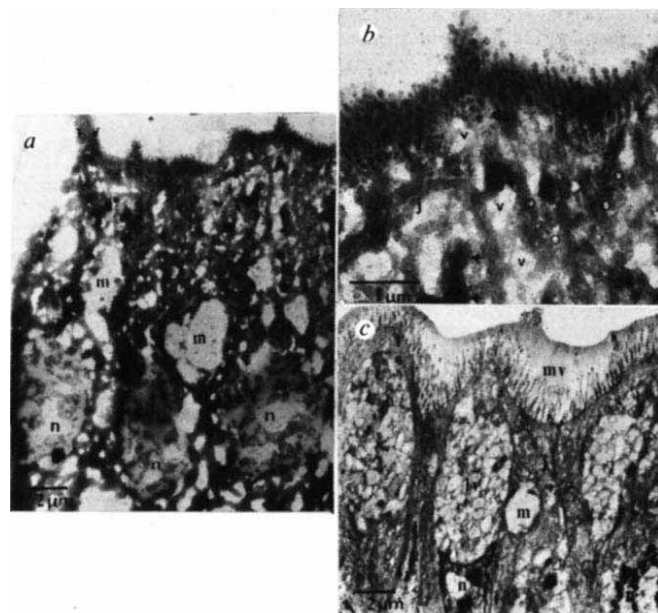


Fig. 1 *a*, Low power electron micrograph of freeze-dried ultrathin frozen section of the regulating epithelium *Otala lactea*. *b*, High power electron micrograph showing the detail in the apical region of the regulating epithelium (from *a*). *c*, Conventionally prepared section of regulating epithelium after fixation and embedding. mv, Microvilli in the apical region; m, mucocyte cells; n, nuclei; j, junctional complexes; v, vesicles (in *b*); lv, (in *c*) laminate vesicles; arrows (*b*) indicate mitochondria; circles (*b*) enclose ice-crystal artefacts.

sections are summarised in diagrammatic form in Fig. 2, and show that in the apical region there is a marked increase in the relative concentrations of both chlorine and potassium, and that there is a significant concentration gradient within the cells of the regulating epithelium from the apical plasma membrane to the nuclear regions at the base of the cells. There is, however, a difference between the 'light' and 'dark' areas of the nuclei: it is not clear at this stage if the 'dark' areas of the nucleus represent the condensed chromatin. There is also a greater variance in the chlorine and potassium data than for the other elements shown in Fig. 2. It may be that this is an indication that these elements are predominantly unbound and that their concentrations are not constant. The lower variances for phosphorus and calcium may be due to the fact that they are at least partially 'bound'.

The silicon levels in the apical region of the regulating epithelium are significantly higher than in the controls ($1,011 \pm 72$ regulating; 685 ± 68 controls). A concentration gradient is established similar to that shown for chlorine and potassium. This cannot be an artefact due to silicon contamination from the glass knives because cutting with diamond knives does not reduce the levels of silicon observed. It cannot be due to contamination from silicon in the oils of the microscope vacuum system because this element is absent from the oils used in our instrument. X-ray microanalysis has also established that there is no silicon in the carbon used to evaporate

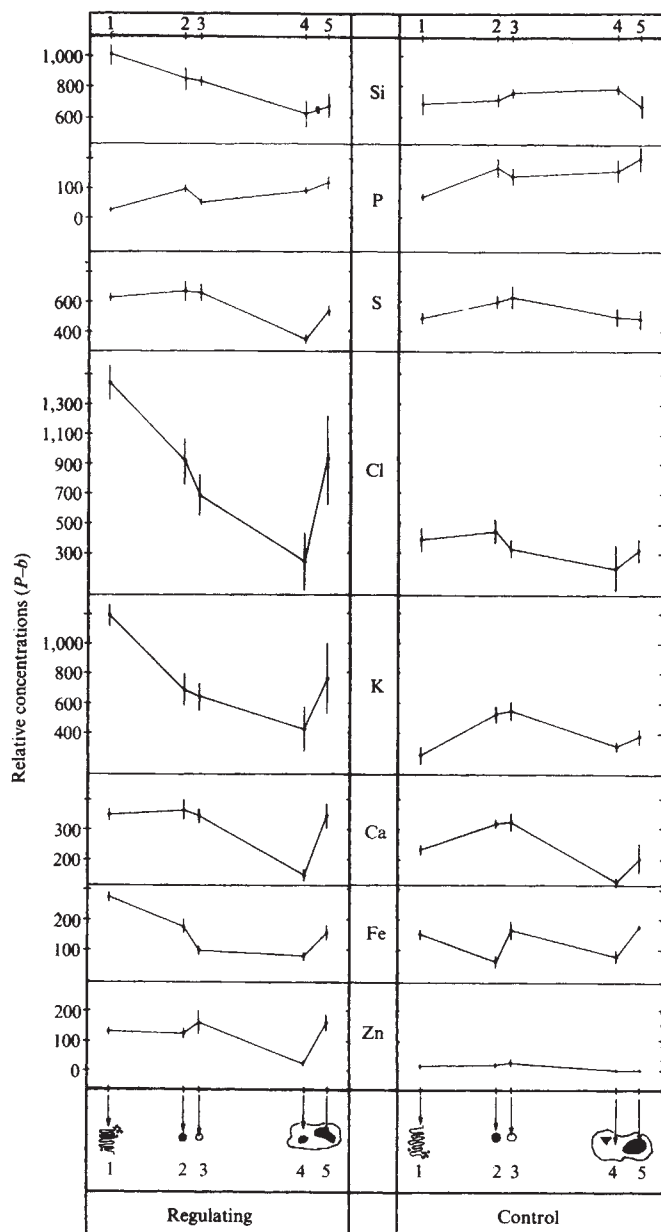


Fig. 2 Elemental composition (by volume) of various elements at four levels with the epithelial cells in regulating and control snails (*Otala lactea*). 1, Apical microvilli region; 2, mitochondria; 3, adjacent ground cytoplasm below each mitochondria; 4, nucleus 'light areas'; 5, nucleus 'dark areas'. The variances shown are the standard errors of the means and reflect true biological variation rather than errors in acquiring the analytical data. No figures are given for sodium because the sodium levels in general were too low to reflect any significant trends. When potassium levels were high, however, the sodium levels were low. In a few examples the higher sodium levels were always accompanied by lower potassium.

the 20–30-nm layer of carbon over the sections. And it cannot be due to electrons being scattered by the denser organelles, thereby generating silicon X-rays within the lithium-drifted silicon detector, because the mitochondria show a greater electron density but not the highest level of silicon. We feel therefore that silicon may have a greater biological significance than has been assumed.

The changes in concentration of iron and zinc between regulating and control epithelium indicates that these elements are related to fundamental physiological processes within the cells. The increased concentration of zinc, for example, may be related to its role as an enzymatic cofactor associated with carbonic anhydrase, which is known to be present in mantle epithelial tissue⁸.

These preliminary results have shown that X-ray microanalysis of freeze-dried ultrathin (130 nm) frozen sections is sufficiently

sensitive to detect physiologically significant changes in the concentration of elements at the subcellular level. Further measurements on the elemental composition of structures within these cells are being made and these results will be published in the near future.

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Right-left asymmetry of myelin development in epiretinal portion of rabbit optic nerve

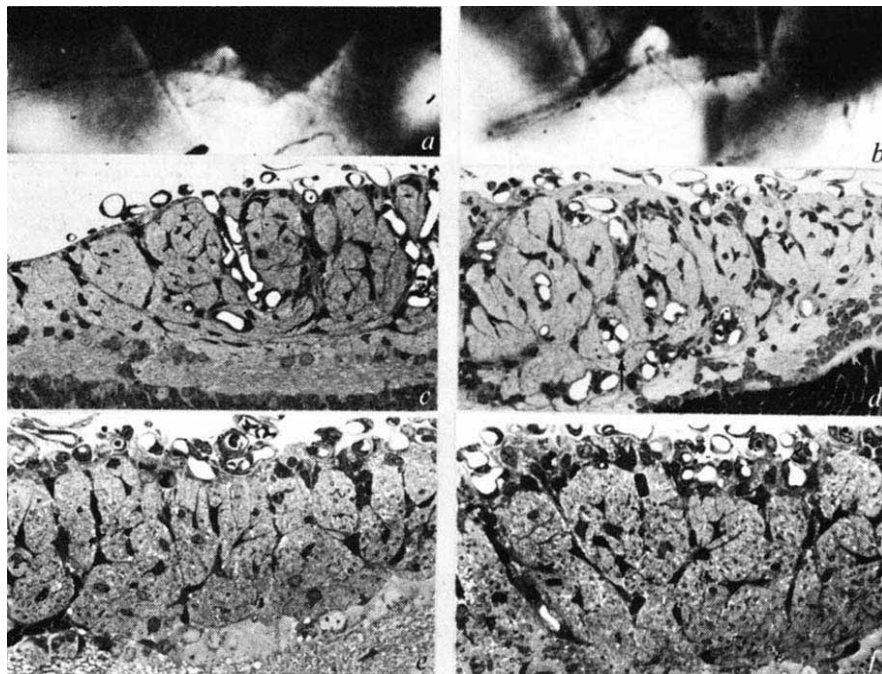
DOMINANCE is the priority or preferential activity of one member of any bilateral pair of structures in the body. The phenomenon of dominant hand, foot and eye for example is well known in humans and this basic sidedness is thought to be cerebral in origin. Based on experiments on audiogenic seizures evidence has been produced for an asymmetry of cerebral function in certain inbred strains of mice¹. In other animal species dogs are known to have preference of paw, and right-left eye dominance in the visual cortex of cats has been demonstrated². Various theories have emerged to explain ocular dominance^{3,4}. It is generally accepted that this is not associated with significant structural differences between the two halves of the brain⁵. Apart from morphological evidence for left-right asymmetries in temporal lobes of speech regions⁶, very little evidence for anatomical differences has been produced in humans or other animals. The rabbit retina is unique in having bundles of myelinated nerve fibres extending equatorially from the optic disk, which are unmyelinated at birth but in which myelination begins at 9–10 days of age⁷. I present here results which indicate that the sequence of myelination is asymmetrical between the right and left eyes, one being dominant.

Rabbits are born with closed eyes which open on the 10th day after birth. I noted that 19 out of 24 rabbits opened the right eye first followed by the left several hours later while the remaining 5 rabbits opened the left eye first.

The optic nerve enters the retina 3–4 mm above the mid-brain from the optic head and extends nasally and temporally into two bands of 'medullary rays'. The epiretinal strips were dissected after whole body perfusion with 4% glutaraldehyde⁷ and immersed in 1% osmic acid. The eye which opened first showed blackening of the fibres at a comparatively faster rate than the other eye (Fig. 1a, b). These epiretinal strips were embedded as previously described⁷.

Serial 1 μ m sections were cut from nasal to temporal side of the epiretinal strips of both right and left eye from 24 animals; 12 more rabbits were also included in this study. The sections from the 9-d-old rabbit showed only a few myelinated nerve fibres close to the disk of one eye, while no myelinated fibres were present in the other (Fig.

Fig. 1 *a, b*, Epiretinal portion dissected from 9-d-old rabbit. Left (*a*) and right (*b*) eye photographed 15 min after osmication. Note the intensity and size difference of the L-R strip. *c, d*, 1 μ m section from 9-d-old rabbit of left (*c*) and right (*d*) eye epiretinal strip through the optic disk stained with toluidine blue showing a few myelinated fibres in the right strip (*d*) (arrow). $\times 160$. *e, f*, Left (*e*) and right (*f*) eye epiretinal strip section through the optic disk of 10-d-old rabbit. Note the dominant myelinated fibres in right (*f*) eye. $\times 160$.



1*c, d*). The right eye was usually more advanced than the left.

From the 10th day onward, both eyes show myelinated fibres, but where the right eye opened first, the number of myelinated fibres was much greater than in the left eye which opened several hours later (Fig. 1*e, f*). In cases where the left eye opened first the left eye was more advanced in the number of myelinated fibres. This anatomical feature of myelin dominance was difficult to see later than 22 d after birth. The nasal and temporal halves of the strip of each eye are also asymmetrical in both size and development of myelin sheaths, the latter side being dominant.

Examination of 1 μ m sections from right and left optic nerves from 1–3-d-old rabbits also showed that earlier myelination of the nerve fibres in the right eye occurred in about 75% of the animals, and earlier myelination of the left was seen in 25%.

In the adult rabbit the anatomical asymmetry which exists during early development could not be observed morphologically. It was, however, demonstrated by light stimulation studies.

It is likely that the earlier onset of myelination in one eye is associated with the dominant brain hemisphere, which during development would most probably show morphological asymmetry, possibly one side (half of body) developing at a comparatively faster rate rather than the size difference between the two halves of the hemisphere. The ratio 3:1 between the right-left dominance suggests that the mechanism is genetically controlled.

It is tempting to draw a comparison between the asymmetry in rabbit and man in that the cortical dominance may be due to early maturation of one half of the hemisphere and nerve fibres arising from it rather than neurone size.

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New type of representation for mapping chain-folding in protein molecules

CONFORMATIONAL details of small molecules can easily be visualised in the usual stereo diagrams used by crystallographers, but such details cannot be readily visualised for protein molecules made up of thousands of atoms. For such large molecules special types of representations become necessary for depicting their three-dimensional structure, in the form of two-dimensional diagrams. In view of this, many attempts to represent the conformational details in protein molecules, have been reported^{1–7}. I present here a new method for mapping the folding of the backbone polypeptide chain of protein molecules. This representation brings out all the features of protein folding, with no degeneracy caused by depicting the conformational details, and is useful in readily recognising all the secondary structures, without ambiguity, as one progresses along the chain.

The backbone conformation of a protein chain can be completely specified by the twin dihedral angles (ϕ, ψ)⁸ since the 'peptide unit' can be taken to be planar for all practical purposes. (There are some indications that the peptide unit is nonplanar^{9,11}, but the deviations from planarity can be considered to be very small when compared to the full range of variations for the dihedral angles ϕ around the single bond N—C α and ψ around C α —C.) It has become customary to plot the (ϕ, ψ) values at each C α -atom of a protein molecule in the Ramachandran map to show how the dipeptide conformations conform to the limits of the hard-sphere and soft-sphere models in the (ϕ, ψ) map¹². But the details of the secondary structures as one progresses along the chain are not readily available in this representation. In order to achieve this, some single parameters have been developed to be used in chain plots^{5,6}, but there is an inevitable degeneracy in these chain plots, since many conformations will give rise to the same value for such a single parameter representing a particular residue.

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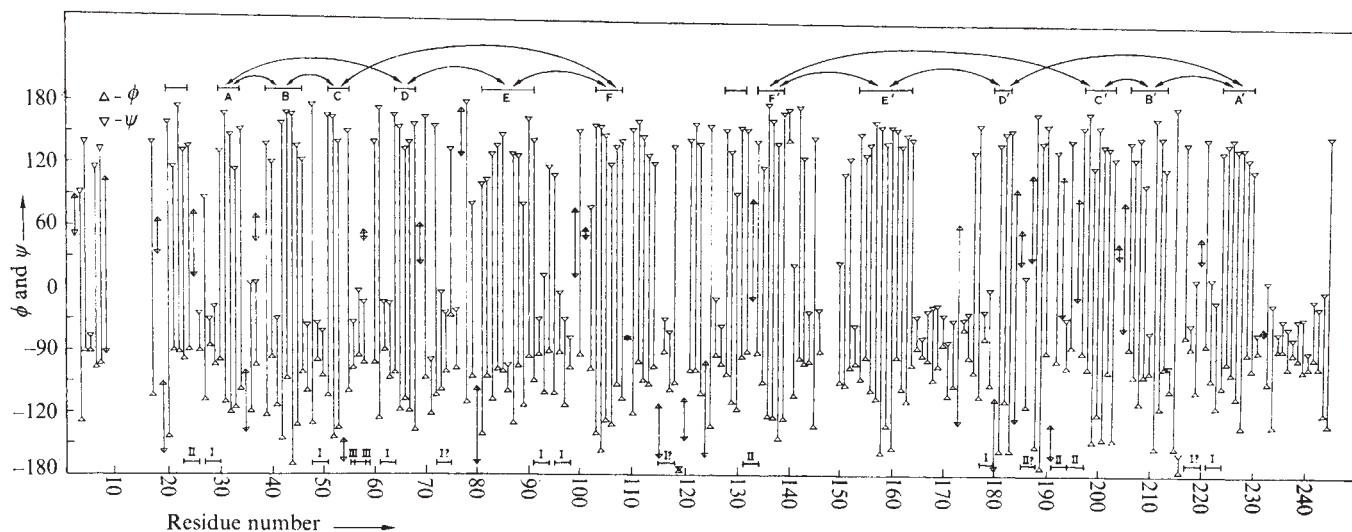


Fig. 1 The new type of mapping for the protein molecule α -chymotrypsin. Δ , ϕ values; ∇ , ψ values. I, II and III represent the three types of bends. The arrow-headed bows indicate the bonding mates of the β -stretches A, B and so on. For further details see text.

The following chain representation overcomes the difficulties mentioned above, and seems to give a good overall picture of the folding of the protein molecule as one progresses along the chain.

The residue numbers are sequentially taken along the X-axis and the corresponding ϕ and ψ values are plotted along the ordinate with different symbols. Symbol Δ is chosen for ϕ and, ∇ for ψ . For proper visual effect these two symbols for a given residue are joined by a vertical line. This kind of chain representation for mapping of protein folding is shown in Fig. 1 for the case of α -chymotrypsin (ref. 13).

In this representation the α -helices are seen as stretches of short vertical lines around -55° on the ordinate (see, for example, residues around 240). The β -stretches are seen as stripes of long vertical lines encompassing almost the whole range of the ordinate (see for example, residues around 225).

The three types of bend conformations^{13,14} or loops can be at once recognised and identified in this kind of map. The residues corresponding to the three types of loops are marked as I, II and III at the bottom of Fig. 1; characteristically, loop I has two short lines sandwiched between two long lines (see for example residues 27–30); loop II has a similar pattern, but the third residue has ψ value less than the ϕ value (see for example residues 23–26). Loop III is simply a part of 3.0_{10} -helix and an example is seen in the residues 55–59. It should be noted that changes from these characteristic patterns would give tell-tale evidence of distortion in the loops. In Fig. 1 such distorted loops have been marked with a question-mark and these agree well with the observations of Birktoft and Blow¹³.

Important feature of the folding in α -chymotrypsin are the two approximately similar domains, each having a 'barrel' of anti-parallel β -sheets, termed cylinder I and II by Birktoft and Blow¹³. Each cylinder is made up of six anti-parallel β -stretches, each of which is hydrogen-bonded to two neighbouring β -stretches, and thus forming a barrel. The six β -stretches have been marked by horizontal lines just above the corresponding plots in Fig. 1. They have been labelled A, B, C, D, E, F in cylinder I and A' B' C' D' E' F' in cylinder II, following Balasubramanian¹⁵. The bonding mates of each of the β -stretches have also been indicated by arrow-head 'bows'. The broad similarity between the patterns in this mapping for cylinder I and II is obvious.

Shotton and Watson¹⁶ found reverse internal homology in tosyl elastase and extended the idea to α -chymotrypsin which belongs to the same family of proteins—the serine proteases. Nishikawa and Ooi¹⁷ also contend that the primary structures of the two similar domains correspond to each other in the opposite sense; that is, residues 29 to 112 correspond to residues

230 to 133 in an approximate way. Hence the β -stretches involved in the cylindrical domains have been appropriately labelled¹⁵, and an approximate mirror symmetry can be seen in the map (Fig. 1), with the mirror situated near residue number 122. But a closer look may justify a translational symmetry too. It is readily seen that for the β -stretch of residues 20–24, there is a corresponding one of residues 128–132 (these sequences of residues can in a way be called β -stretches, though there are no regular hydrogen bonds with a neighbouring extended conformation); these stretches precede the stretches of residues forming the similar domains. However, in the distorted β -stretch B, there is a 'break' at residue 41 (a short vertical line) and correspondingly, there is one at 210 in the distorted β -stretch B'. The paradoxical existence of both the approximate mirror symmetry and translational symmetry in the map arises because there is already an approximate mirror symmetry in the stretches 29–112 corresponding to cylinder I and 133–230 corresponding to cylinder II (a mirror could be 'placed' near residue 60 and another near residue 190). Due to the distortions in the similar domains¹³, one cannot resolve the question unequivocally. Nevertheless, this type of mapping is very useful for observing such intrinsic details of chain folding.

The method of mapping chain folding in protein molecules presented here seems to have many advantages over many other types of representations reported in the literature, and can be used as a uniform and stereotyped 'finger printing' for all protein molecules.

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